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Optimization of Conditions for Heat Pretreatment and Enzymatic Predigestion of DDGS for Pigs

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OPTIMIZATION OF CONDITIONS FOR HEAT PRETREATMENT AND ENZYMATIC PREDIGESTION OF DDGS FOR PIGS

BY

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THESIS ACCEPTANCE PAGE

KEVIN JEREZ-BOGOTA

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Yo fuerte, yo exaltado, yo anhelante,

opreso en la urna del día,

engreído en mi corazón,

ebrio de mi fantasía,

y la eternidad adelante,

adelante...

adelante...

- *Porfirio Barba Jacob, "Síntesis"*

A mi familia y amigos

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CONTENTS

ABBREVIATIONS

AA	Amino acid
ABE	Acetone, butanol and ethanol
ADF	Acid detergent fiber
AFEX	Ammonia fiber explosion
AIC	Akaike information criterion
AID	Apparent ileal digestibility
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
ATTD	Apparent total tract digestibility
CDS	Condensed distillers solubles
CP	Crude Protein
CV	Coefficient of variation
Cys	Cysteine
DDGS	Distiller's dried grain with solubles
DE	Digestible energy
DF	Dietary fiber
DM	Dry matter
DWG	Distillers wet grains
EE	Ether extract
GE	Gross energy
GIT	Gastrointestinal tract
Gln	Glutamine

Glu	Glutamic acid
Gly	Glycine
HI	Heat increment
His	Histidine
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
IDF	Insoluble dietary fiber
Ile	Isoleucine
IVD	In vitro digestibility / disappearance
IVDDM	In vitro degradation of dry matter
Leu	Leucine
LHW	Liquid Hot Water
LS	Least squares
Lys	Lysine
Met	Methionine
mm	millimeter
MPD	Multienzyme predigestion
NDF	Neutral detergent fiber
NE	Net energy
NSP	Non starch polysaccharides
NSPase	Non starch polysaccharides degrading enzymes
OM	Organic matter
Phe	Phenylalanine
Pro	Proline
RDS	Rapid digestible starch

RS	Resistant Starch
RS	Resistant starch
SBM	Soybean meal
SDF	Soluble dietary fiber
SDS	Slowly digestible starch
Sec	Selenocysteine
SEM	Standard error mean
Ser	Serine
SID	Standardized ileal digestible
TDF	Total Dietary Fiber
TEMP	Temperature
TGP	Total gas production
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
US	United States
Val	Valine
VFA	Volatile fatty acids
WS	Whole Stillage
°C	Celsius degrees
°F	Fahrenheit degrees

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ABSTRACT

OPTIMIZATION OF CONDITIONS FOR HEAT PRETREATMENT AND ENZYMATIC PREDIGESTION OF DDGS FOR PIGS

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The high fiber content of corn dried distillers' grains with solubles (DDGS) can limit its utilization in swine diets. Pretreatment with heat and enzymatic predigestion of whole stillage (WS; slurry material that is dried into DDGS) can alleviate negative effects of dietary fiber and can improve digestive and fermentation characteristics of the feedstuff. However, optimal time and temperature of the heat pretreatment of WS, and best enzymes for predigestion of WS have not been identified. Experiments were conducted to identify optimal conditions for heat pretreatment and multienzyme predigestion of WS for pigs. First experiment was conducted to identify optimal temperature and time for later use in large-scale pretreatment of WS for in vivo studies. The treatments were untreated WS, and WS that was pretreated (at 70 psi) for 10, 20, or 30 minutes and at 100, 120, 140, or 160°C in a 3 × 4 factorial arrangement. Sub-samples were subjected to porcine in vitro digestion and fermentation. An increase in pretreatment temperature linearly and quadratically increased ($P<0.05$) in vitro dry matter digestibility (IVDDM) by 11%, and linearly increased ($P<0.05$) total gas production (TP) by 13%. Response surface analysis indicated that maximum IVDDM resulted from pretreatment time of 20-30 minutes and highest pretreatment temperature, whereas maximum TP resulted from pre-treatment time of 10–20 minutes and highest pre-treatment temperature. Thus, 160°C and 20 minutes were the apparent optimal pretreatment temperature and time. Second experiment was conducted to identify best

multi-enzyme for large-scale predigestion of WS. Four WS samples were obtained from 4 different sources. Half amount of WS from each source was pretreated at 70 psi and 160°C for 20 minutes. Untreated and pretreated WS samples from each source were divided into 4 sub-samples. Eight treatments were applied to the sub-samples within source. The treatments were untreated and pretreated WS undigested or predigested with 1 of 3 multi-enzymes (ME1, ME2, and ME3) for 12 hours. The ME1; ME2; and ME3 respectively contained xylanase, β-glucanase, cellulase, mannanase, protease and amylase; xylanase, α-galactosidase, and celullase; xylanase, cellulase, β-glucanase and mannanase. Sub-samples were subjected to porcine in vitro digestion. Multienzyme improved (P<0.05) IVDDM of untreated WS and heat pretreated WS by means of 9.1 and 6.8 percentage points, respectively. However, predigestion of pretreated WS with ME3, compared with ME2, resulted in lower (P<0.05) magnitude of improvement in IVDDM (4.8 vs. 9.0 percentage points); the magnitude of improvement in IVDDM for ME1 (6.7 percentage points) did not differ from that for ME2 or ME3. Thus, ME1 and ME2 were the best multienzyme complexes for predigestion of WS. Last experiment determined standardized ileal digestibility (SID) of amino acids and digestible energy (DE) value for WS that was pretreated or predigested on large-scale. Ten ileal-cannulated pigs were fed 5 diets in a replicated 5×5 Latin square design. Diets were cornstarch-based with DDGS, untreated WS, ME2- predigested WS or heat-pretreated WS; and nitrogen-free diet. Untreated WS and DDGS did not differ in NE. Predigestion increased (*P<0.05*) SID of lysine and NE of WS. Heat pretreatment reduced (P<0.05) SID of lysine and NE value for WS. Predigestion of WS with ME2 can enhance nutritive value of resulting DDGS. Predigestion with enzymes

of WS can be an attractive option for improving DDGS nutritive value for pigs. Optimal conditions for heat pretreatment of WS still need to be adapted for large-scale pretreatment.

GENERAL INTRODUCTION

As the world population is growing, the increasing demand for food, fuel and fiber is one of the most crucial challenges facing society in the current time. For instance, the energy consumption in the United States (**U.S.**) by 2018 was almost three times that of 1958 (EIA, 2019). Most of that energy comes from fossil fuels (petroleum, natural gas, and coal); nevertheless, these sources of energy are non-renewable and hence great attention has been paid to renewable sources of fuel from agricultural products. As a result of these trends, renewable fuel sources have been explored from agricultural products such as miscanthus, switchgrass, sugar cane, rapidly growing tree species, and corn (Mumm et al., 2014).

The U.S. has a well-developed production scheme for corn, hence this crop has become the most important crop used for the bioethanol production (95% of ethanol produced in US is derived from corn) and the production of the combustible has grown by approximately 40% since 2002 (Zeng et al., 2017; USDA, 2019). Additionally, the introduction of policy such as the Renewable Fuel Standard in 2005, which set minimum quota for the use of renewables, including ethanol, has incentivized the corn grown for ethanol production at expense of corn grown for animal feed and other residual uses (EIA, 2019). Consequently, in 2018 the production of ethanol for fuel was at its historic maximum with more than 16 billion gallons (RNF, 2019).

In addition to ethanol, the corn-based bioethanol scheme generates high amounts of co-products, including distiller's dried grain with solubles (**DDGS**), corn gluten feed, and corn gluten meal (Mumm et al., 2014; Smith et al., 2017; AFDC, 2019). In 2018, the U.S. ethanol industry generated more than 40 million metric tons of corn DDGS, which is

about 4 times the amount that was produced 15 years ago (RNF, 2019; USDA, 2019). Along this increment in production, a relatively large amount of research has been conducted in the last 15 years regarding the use of corn DDGS for feeding livestock (see **Figure 1**). Thus, the nutritive value and dietary inclusion limits have been reported for several livestock species (Klopfenstein et al., 2008; ŚWiĄTkiewicz and Koreleski, 2008; Schingoethe et al., 2009; Stein and Shurson, 2009) and it has been proposed as alternative for replacement of corn and soybean meal in livestock diets due to its relatively high energy and protein content (Arora et al., 2010; Mumm et al., 2014; Lewandrowski et al., 2019).

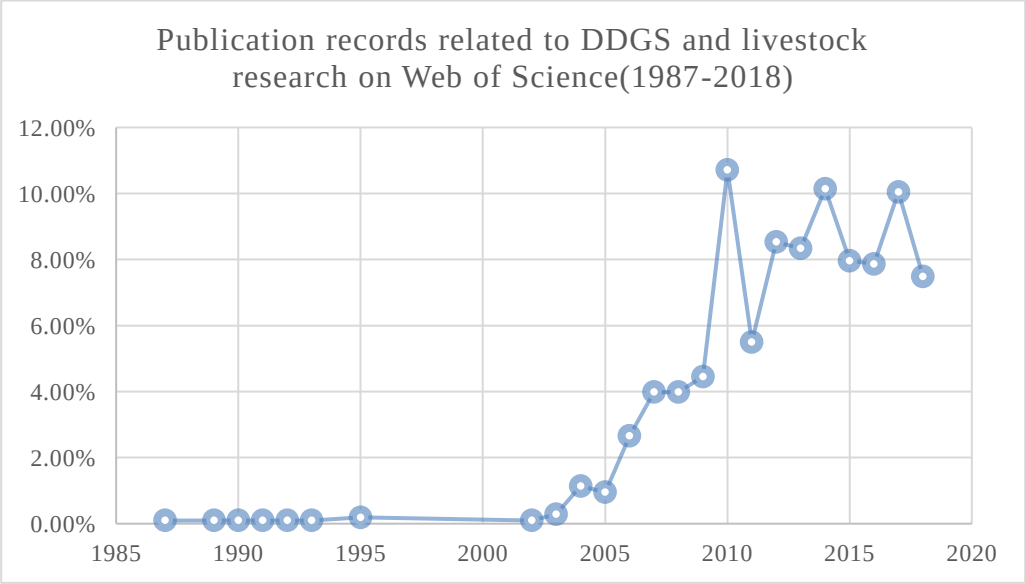

Figure 1. Publication records related to DDGS and livestock research. Based on Web of Science search, words string: (AGRICULTURE) AND TOPIC:(DDGS AND (PIGS) OR (POULTRY) OR (DAIRY) OR (BEEF)))

Despite the fact that corn DDGS has a high gross energy (**GE**) content compared to that of corn (5,429 vs. 4,454 kcal/kg DM; NRC, 2012), the efficiency of utilization of energy in DDGS by monogastrics such as pigs and poultry is lower than that of the corn grain (Stein and Shurson, 2009). For instance, the GE value of corn is 3,933 kcal/kg and its net energy (**NE**) value is 2,672 kcal/kg, while the GE of corn DDGS is 4,849 kcal/kg

and its NE value is 2,384 kcal/kg (NRC, 2012). Thus, the NE value compared with GE value is lower by 32% for corn and by 51% for corn DDGS. Since in monogastrics, fat is a very efficient energy source followed by starch whereas fiber and protein are used with much less efficiency, the low efficiency of utilization of energy in DDGS is due to its high level of crude protein (**CP**) and dietary fiber content (Kerr and Shurson, 2013; Knudsen, 2014; Pedersen et al., 2014). Along with it, non-starch polysaccharides (**NSP**) present in dietary fiber can reduce utilization of other nutrients in monogastric animals (Choct et al., 1996; Jha and Berrocoso, 2015). Finally, the manufacturing process of DDGS includes thermic treatments that affect protein quality as a result of Maillard reactions (Adeola and Ragland, 2016). Therefore, DDGS are mainly used in ruminant diets, since the dietary fiber level and protein quality limit the utilization of corn DDGS by swine and poultry (Stein and Shurson, 2009; de Vries et al., 2012).

It is of great interest for the swine and poultry industries to overcome the limitations on the use of corn DDGS in diets. Disruption of the fiber structure by various means has been proposed as one of the methods for alleviation of negative effects of dietary fiber in DDGS. For instance, Swiatkiewicz et al. (2016) reviewed the effects of adding NSP-degrading enzymes[1] (**NSPase**) to DDGS-based diets for pigs and poultry, and observed that the efficacy of NSPase on nutrient utilization has not been consistent due to various factors including enzyme purity, optimal enzyme pH and temperature ranges, and resistance of enzymes to degradation by proteolytic enzymes in the gastrointestinal tract (**GIT**). Additionally, de Vries et al. (2012) reviewed the effects of processing technologies

[1] Including Xylanase, Cellullase, B-glucanase, pectinase and other enzymes that targets components of the cell wall structure of plant cells, usually refer as NSPase or sometimes refer as carbohydrases although such category would include amylases. Recent literature refer to NSPase when more than one fiber degrading enzymes is used for treatment.

with and without NSP degrading enzyme addition to monogastric diets, and observed that processing technologies (e.g., pelleting, extrusion, and milling) resulted in a greater digestibility coefficients, but in some cases increased negative characteristics such as viscosity. Nevertheless, the addition of NSPase seems to overcome these negative effects in some cases, and a combination of technologies might result in additive or synergistic effects on nutrients utilization since processing might increase the accessibility of NSP to enzymes.

Pretreatment technologies have also been used for improving DDGS quality. For instance, Kim et al. (2008) investigated the effects of ammonia fiber expansion pretreatment of corn DDGS and observed lower fiber and higher protein content in pretreated DDGS than in untreated DDGS. Recently, Zangaro et al. (2018) determined the effects of combing heat pretreatment and enzymatic predigestion of whole stillage (**WS**; slurry material that remains after distillation of fermented corn mash, which is subsequently centrifuged and dried into DDGS) on porcine in vitro digestion and fermentation characteristics, and observed increased digestibility of WS due to the pretreatment and predigestion. However, optimal time and temperature of the heat pretreatment of WS, and best NSPase for predigestion of WS have not been identified. Also, the effects heat pretreatment and enzymatic predigestion of WS on *in vivo* nutrient utilization have not been determined. Thus, the general objective of this thesis research was to fill this gap in knowledge.

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1. LITERATURE REVIEW: Nutritive value of corn DDGS in swine diets and methods for improvement of utilization

1.1. CORN AND ETHANOL PRODUCTION

Corn is the most produced cereal grain in the world and is used for livestock feeding, manufacturing of human food products, and other industrial products including ethanol (FAO, 2019). The US is the major producer of corn in the world; more than 365 million metric tons of corn were produced in US in 2018 (NCGA, 2019). Although several cereal grains can be used for production of bioethanol; corn is the main cereal grain used to produce bioethanol in the US, where it is favored by a well-established production scheme. The US is the largest ethanol producer in the world. For instance, more than 16 billion gallons of ethanol were produced in US in 2018 (AFDC, 2019). Approximately 56% of corn grown was utilized by ethanol industry (see **Figure 1.2**). Consequently, there is an increasing availability of co-products from the ethanol industry.

1.2. CORN BIOETHANOL AND DDGS PRODUCTION PROCESS

There are three industrial methods for converting corn into ethanol: wet milling, dry milling, and dry grind processing. During ethanol production by wet milling method, corn is fractionated into four components (i.e., starch, germ, fiber, and protein) with the goal of obtaining starch that is used to produce purified substances such as glucose, high fructose corn syrup, ethanol, and other chemicals. During ethanol production by dry milling method (the term dry milling is sometimes used erroneously to describe the dry-grind process) corn is processed through an abrasion (degermination) that splits the kernel into pericarp (bran), germ and flaking grits (endosperm pieces; Rausch and Belyea, 2006). The principal product of dry milling is the flaking grits, which are used for production of breakfast cereals and ethanol. Lastly, the dry-grind process is designed to subject the entire corn kernel to

fermentation after a grinding process that decreases particle size in order to maximize ethanol yield. In the US, about 90% of the ethanol production is by dry-grinding process (US-GRAIN-COUNCIL, 2019). Therefore, dry-grind method will be further discussed in the rest of this section. Nonetheless, a complete description of the other two methods can be found in Rausch and Belyea (2006) and Ray and Ramachandran (2018).

The main co-product of the dry-grind method of ethanol production from corn is corn DDGS. The US ethanol plants produced 41.3 million metric tons of corn DDGS in 2018 and from that amount, approximately 15% was used for feeding swine (RNF, 2019; US-GRAIN-COUNCIL, 2019). During dry grinding, the whole corn grain is first ground using hammer or roller mills followed by the addition of water and enzymes, and incubation of slurry material at temperature of between 60 and 90°C (Liu and Rosentrater, 2016). Amylase is added to the slurry material to hydrolyze starch into maltose. Maltase is added to the slurry material to hydrolyze maltose into glucose, which then undergoes alcoholic fermentation. Nonetheless, other enzymes such as proteases and fiber degrading enzymes might be added as well to increase starch exposure to amylase (Brown and Brown, 2013). Subsequently, the digested slurry material is subjected to fermentation. The fermentation reaction is achieved by incubation of the digested slurry material with the yeast at 32-35°C, which is the optimum temperature for the metabolism of the yeast. The yeast convert glucose into ethanol and carbon dioxide. The most used species of yeast for ethanol fermentation is *Saccharomyces cerevisiae* (Jansen et al., 2017). The fermentation process yields 2 moles of ethanol and 2 moles of carbon dioxide from 1 mole of glucose (see Equation 1). After fermentation, the raw ethanol in the slurry material is distilled and purified to at least 99.8% ethanol (Riazi and Chiaramonti, 2017).

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

Equation 1. Chemical reaction of alcoholic fermentation of sugars.

The remaining material after alcoholic fermentation and distillation is known as whole stillage (**WS**), which has 5 to 15% of solids and is composed of the non-fermentable components of corn grain such as protein, fiber, lipid and minerals in suspended and dissolved states. The WS is subjected to dehydration process that involves a series of centrifuging, drying and combination of streams that results in the final product known as DDGS. The process begins with a centrifuging of the WS into in 2 fractions: thin stillage and wet cake. Thin stillage contains high amounts of the water-soluble solids, whereas, wet cake (also known as distillers wet grains: **DWG**) contains the suspended solids that were removed from the WS (Liu and Rosentrater, 2016). The thin stillage is passed through a multiple-effect evaporator system to increase the solids content to between 25 and 55%. At this stage the evaporated fraction is known as condensed distillers solubles (**CDS**), which is also commonly known as "syrup" in the corn-ethanol industry (Brown and Brown, 2013). Additionally, most of the US ethanol plants nowadays extract oil from thin stillage, which is then commercialized separately; complete description of the current method used for this purpose can be found in 4th edition of the U.S. Grains Council DDGS User's Handbook (US-Grains-Council, 2018).

Finally, the two fractions (CDS and DGW) are combined and undergo a drying process. This drying process is energy-intensive; it can consume a third of the total energy used by the ethanol plant (Gallagher, 2015). The predominant drying system used in the US for DDGS production is rotary drum. The process starts with the streams of CDS and DWG that are passed to a mixing chamber, screw conveyor, or paddle mixer with a portion of

freshly dried DDGS. The proportion of each fraction varies between plants; nevertheless, the blend is generally prepared in such a way that the solids content of the mixture is about 65% prior heat application in the dryer (Liu and Rosentrater, 2016). The mixture is subjected to heat in the drying chamber where the temperatures can be over 500 °C (932 °F) in the inlet and over 100 °C (212 °F) in the discharge. The product enters the chamber and move in the same direction as the airflow inside the dryer. At the discharge, the DDGS temperature would be slightly lower than the chamber (about 90 °C) with a dry matter (**DM**) of between 88 and 90%. As previously mentioned, between 30% to 50% of the material is routed back to the mixer where it is blended with incoming DWG and CDS, whereas the rest is conveyed to storage for later use as livestock feed (Liu and Rosentrater, 2011). Residence time in rotary dryers may vary from 10 to 20 minutes, although this time may be as long as 60 minutes for material with a high moisture content. The machinery and methods involved in the drying process have an important impact on the final composition and characteristics of the resulting DDGS. For instance, the proportion of DWG, CDS and recycled DDGS entering the dryer affect the final composition of the resulting DDGS (Liu, 2009a). Additionally, the drying temperatures, the air flow rates in the drying chamber and the drum rotation speed can have an impact on the nutritional quality of the resulting DDGS because of the effect of Maillard reactions (Liu and Rosentrater, 2016). Some other techniques for drying include ring drying system, superheated steam dryers, steam tube drying systems and compression dryer system. A complete description of these systems can be found in Liu and Rosentrater (2016) and US-Grains-Council (2018).

1.3. NUTRIENT COMPOSITION OF MODERN CORN DDGS

1.3.1. Reference Values for Corn DDGS Composition

The latest version of the National Research Council: Nutrient Requirements of Swine (NRC, 2012) classified corn DDGS based on its oil content. These classes are: corn DDGS > 10% oil; corn DDGS 6 - 9% oil and corn DDGS < 4% oil (NRC, 2012). At the time of its publication, the type of DDGS with most information was the one classified as corn DDGS > 10% oil. The main difference between the three types of corn DDGS is related to its oil content and therefore, it is expected that as the oil content of the ingredient decreases, the ME and NE values will follow. As most ethanol plants have already introduced oil extraction technology, it is worth stressing that modern corn DDGS is primarily classified as corn DDGS 6 - 9% oil. There is therefore a need to update the published reference values for corn DDGS. Because, approximately two-thirds (weight basis) of corn is converted into carbon dioxide and ethanol during fermentation in the ethanol plant, a concentration of about three times the level of the other nutrients is expected in the DDGS relative to corn (Bothast and Schlicher, 2005). The following sections will discuss available data on nutrient composition of corn DDGS relative to that of corn grain. The following major nutritional components would be discussed separately; carbohydrates, protein and amino acids (**AA**), lipids, minerals, vitamins and finally an overview of how the composition of DDGS determine its energy value for pigs.

1.3.2. Carbohydrates Components in Corn DDGS

1.3.2.1. Major Carbohydrates in Cereal Grains. Carbohydrates are often classified using several nomenclatures. However, the most common classification is based on their molecular size. This criterion classifies carbohydrates into monosaccharides,

oligosaccharides, and polysaccharides (BeMiller, 2018). Monosaccharides are carbohydrate molecules that cannot be broken down by hydrolysis into simpler carbohydrate molecules, thus they are often referred as “sugars” or “simple sugars”. Chemically they are subdivided into aldoses (have aldehyde as functional group) or ketoses (have ketone as functional group). Sugars are commonly classified according to the number of carbon atoms in their backbones; designated with prefixes such as tri-(3), tetr-(4), pent-(5), hex-(6), etc. Further classification designates these molecules based on the stereochemical configuration: as D (dextro) and L (levo). Finally some sugars can exist in three structural forms: the open chain, the alpha (α) cyclic form, and the beta (β) cyclic form (see Figure 1.3). For example, glucose is an aldose with six carbons (hexose) that most commonly (99% of the times) exist in cyclic from (66% β and 33% α) and occurs widely in nature as the D-glucose isomer (Dilworth et al., 2017).

Oligosaccharides are molecules that consists of a few (2 to 10) monosaccharide units that are joined by glycosidic linkages. These molecules do not commonly occur in nature as oligosaccharides; they are the result of hydrolysis of polysaccharides into smaller units by either acid- or enzyme catalyzed hydrolysis (see Figure 1.4; (BeMiller, 2018). Oligosaccharides are also commonly part of glycolipids and glycoproteins when bound to lipids (O-glycosidic link) and AA (N-glycosidic), respectively (Dilworth et al., 2017).

Polysaccharides are carbohydrate polymers (or macromolecules) that are composed of more than 10 units monosaccharides, and yield monosaccharides or related compounds upon hydrolysis. Polysaccharides are classified into 2 groups: storage polysaccharides and structural polysaccharides. Storage polysaccharides serve as energy reserves in plants (e.g., starch and inulin) or animals (e.g., glycogen). Structural polysaccharides form rigid

protective structures in plants (e.g., cellulose and pectin) and animals (chitins; Dilworth et al., 2017). An important aspect to note regarding polysaccharides is that they differ not solely by the type or diversity in individual residues of monosaccharides, but also by other aspects such as the molecular weight, the nature of chains formed (linear without or with branches) and the glycosidic bond involved (α or β), as well as the position of condensation (1-2,1-1,1-4,1-6). A classic example of this diversity consists of the comparison between starch and cellulose. Although both starch and cellulose are formed of merely glucose units, they differ structurally; cellulose has a linear structures composed of β (1-4) glycosidic, whereas starch can contain linear and branched chains consisting on α(1-4) and α(1-6) linkages (Dilworth et al., 2017).

Starch is the largest storage carbohydrate in the cereal grains. The starch molecule is composed of two types of polymers of D-glucose: amylose and amylopectin. Amylose consists of linear-like α (1-4) linked glucans, whereas amylopectin consists of chains of α (1-4) linked glucans arranged in a highly branched structure with α (1-6) branching links (Copeland et al., 2009).

All polysaccharides different from starch are commonly grouped and categorized as NSP. They can be classified as structural or storage NSP; nevertheless, in cereal grains, they are primarily part of the cell wall structure (Knudsen, 2014). The NSP can be further classified either as soluble or insoluble based on their solubility in water or weak alkali. For instance, cellulose is insoluble, whereas pectins, gums, and β-glucans are soluble (Agyekum and Nyachoti, 2017).

1.3.2.2. Starch Content of Corn DDGS. As previously mentioned, during the dry-grind ethanol production process the starch in corn is hydrolyzed into glucose, which is the

substrate for alcoholic fermentation process that results in ethanol. Therefore, the starch content in DDGS is lower than that of corn. Moreover, since the starch hydrolysis is a technology-dependent process, the characteristics of the resulting DDGS and hence starch in DDGS is partly dependent on the production conditions in the ethanol plant as well as genetics, and growing and storage conditions of the corn grain (Plumier et al., 2015).

Table 1.1 shows starch content of corn grain and the corn DDGS from 11 sources as reported by Pedersen et al. (2014); starch content in DDGS was lower than that of corn by more than 90%. According to Li et al. (2014) some starch granules in corn DDGS are still encapsulated in cells of grain kernel or embedded in protein matrix after milling, which make them physically inaccessible to amylases during early stages of ethanol production. Furthermore, retrograded starch molecules (mainly amylose), complexes of starch with other nonfermentable components, and starch–lipid complexes can be found also in corn DDGS (Li et al., 2014). Most of the residual starch present in corn DDGS is resistant starch (**RS**; Li et al., 2014). Therefore, unlike in corn, it is expected that the starch in DDGS does not substantially contribute to the total valuable energy in the co-product.

1.3.2.3. Non-Starch Polysaccharides Content of Corn DDGS. In corn and DDGS, the most abundant NSP are cellulose and arabinoxylans (Knudsen, 2014). For example, cellulose and arabinoxylans accounted for 21.6 and 48.6 per cent of the total NSP in corn and the DDGS values were 23.3 and 48.7 per cent respectively (Jaworski et al., 2015). Cellulose exists as a pack of microfibers in the cell wall structure, whereas arabinoxylans are composed of a linear backbone of (1→4)-β-D-xylopyranosyl units substituted mainly with α-l-arabinofuranosyl residues to varying degrees (Knudsen, 2014). The arabinoxylans crosslink with lignin components of the plant cell wall (Kang et al., 2019). Compared with

corn, the total dietary fiber[2] (**TDF**) content of DDGS is much higher (13.73 vs 31.35; NRC, 2012). The NSP profile of corn and their respective DDGS was reported by Pedersen et al. (2014), and the values are summarized on Table 1.3. On average the NSP components were approximately 4.5 times greater in DDGS than in corn grain, with the maximum value for soluble xylose and the minimum for soluble glucose. Espinosa et al. (2019) evaluated the nutritional value of low-oil DDGS from 8 different sources; the values for the fiber components are presented in Table 1.3. In this study the TDF levels in all DDGS sources ranged from 36.20 to 41.94%, while the value for corn was of 13.17%. Insoluble NSP levels were much higher than soluble NSP levels in all sources.

1.3.3. Protein and Amino Acids in Corn DDGS

The sources of protein in DDGS are corn and yeast. Yeast is a source of protein in DDGS because during fermentation, it grows and generate cell mass that has greater protein content than corn (36−60 vs. 8.24%; Han and Liu, 2010; NRC, 2012). These protein and other compounds are released when yeast cells undergo autolysis, and it is estimated that yeast contributes approximately 20% of DDGS protein (Han and Liu, 2010). Additionally, Han and Liu (2010) reported changes in the patterns of AA profiles at different points of DDGS production process (Table 1.4), indicating an important contribution of yeast to final protein composition of DDGS. Belyea et al. (2004) did not observe correlation between the protein content of the parent corn and the resulting DDGS; thus, the protein content and AA profile of the DDGS would be more influenced by the process of production of DDGS and the contribution of the yeast biomass. The protein content and specially the AA content of corn DDGS can vary significantly among and within ethanol plants (Zeng et al.,

[2] TDF values, includes NSP, resistant starch and non-digestible oligosaccharides.

2017; US-Grains-Council, 2018). In a review done by Olukosi and Adebiyi (2013) on corn DDGS composition, the crude protein (**CP**) content of corn DDGS varied from 34.7 to 27.9% (CV: 8.5%), and among the indispensable AA in corn DDGS, Lys, Met and Trp were the most variable (CV of 13.1, 12.0, 10.3%, respectively; Table 1.5). In their (Olukosi and Adebiyi (2013) study, power of prediction of the CP value from the AA content was not significant. In a meta-analysis data from 90 studies on AA content data of corn DDGS by Zeng et al. (2017), the variation of the AA content values was generally high (CV above 10%) for all the AA, with the maximum variation value corresponding to Lys (CV:17.85%). More recently, Espinosa et al. (2019) reported lower variation for CP (3.2%) and AA (2.4%); among the indispensable AA the major variation was found for Trp (CV: 6.1%) for low-fat DDGS (Table 1.6). From these studies we can conclude that the new generation DDGS are more homogenous than the old one, likely due to the adoption of new technologies by ethanol plants (US-Grains-Council, 2018).

1.3.4. Lipid Content in Corn DDGS

As previously mentioned, one of the most important changes in modern DDGS includes the reduction in oil content. Several ethanol plants nowadays extract oil from the distiller's grains, which in turn, reduce fat and increase the level of other components such as protein and dietary fiber in the DDGS (US-Grains-Council, 2018). Traditionally, the ether extract content of high-oil DDGS is between 9 and 14%, whereas the ether extract in low oil DDGS is between 5 and 8% (NRC, 2012).

Moreau et al. (2011a) reported the changes in lipid composition of different fractions of corn co-products from dry-grind ethanol production process. The corn kernel contained 3.43 ± 0.10% ether extract, while the resulting DDGS contained 8.67 ± 0.09% ether extract. The total free fatty acids as proportion of ether extract in DDGS was 9.27 ± 0.47%, whereas

the total sterols as proportion of ether extract was 2.34 ± 0.22%; the corresponding values in the corn grain were 2.28 ± 0.02% and 1.79 ± 0.14%, respectively. The reason of the higher content of free fatty acids in corn DDGS is not clear; however, some researchers have attributed this increase to lipase activity in corn or yeasts, continuous pH changes, and the high evaporation and drying temperatures during the production of DDGS from corn grain (Winkler-Moser, 2011; Díaz-Royón et al., 2012). Finally, with regard of fatty acids composition of DDGS, linoleic acid (18:2) is the major fatty acid (it constitute 53.96 to 56.53% of total fatty acids), followed by oleic acid (25.25 to 27.15% of total fatty acids) and then palmitic acid (16.2% of total fatty acids); stearic (1.80–2.34% of total fatty acids) and linolenic acids (1.15–1.40% of total fatty acids content) are minor fatty acids in corn DDGS (Moreau et al., 2011b; Díaz-Royón et al., 2012). No significant differences in the proportional amount of lipids of the corn kernel and the WS after fermentation exist, which indicates that there is a minimal contribution of yeast to the lipid profile of corn DDGS (Moreau et al., 2011a).

Several lipid antioxidants (such as α-tocoferol) are conserved along the process of DDGS manufacturing (Moreau et al., 2011a). Furthermore, lipid antioxidants such as tocotrienols, carotenoids, phytosterols, and ferulate phytosterol are higher in corn distillers' grains extracted oils than in corn germ oil (Winkler-Moser and Breyer, 2011). Shin et al. (2018) reported that DDGS samples had a considerably greater concentration of tocopherols and tocotrienols (lipid-soluble antioxidants) than corn.

Nevertheless, as mentioned before the lipid fraction of corn DDGS contains high level of linoleic acid which is prone to lipid peroxidation (Song et al., 2014). In addition, DDGS is heated at relatively high temperatures during drying, which can accelerate lipid

peroxidation by oxidizing unsaturated fatty acids, which in turn produces oxidized lipids and a series of toxic aldehydes (Prabhu, 2000; Blokhina et al., 2003).

1.3.5. Mineral Content in Corn DDGS

Like other nutrients, the mineral concentration in corn DDGS is assumed to be three times greater than that of the parent grain. Nonetheless, that is not the case for some minerals, likely due to the contribution of yeast, the effect of the combination of different fractions during DDGS production and chemicals that are added to regulate pH during the fermentation process (Batal and Dale, 2003). Therefore, elevated and highly variable levels of minerals is often a concern in the use of corn DDGS. The elevated level of some minerals might lead not only to illnesses, but also to excessive excretion of minerals to the environment. Also, the high variations in mineral content impede precise formulation of diets (Liu and Han, 2011).

Liu and Han (2011) investigated the changes in mineral composition during the production of DDGS from corn, and observed that most minerals, including K, Mg, Cu, Fe, Mn, and Zn increased by about 3 times when corn was processed into DDGS. However, the concentration of other minerals such as Na, S, Ca, and Fe significantly differed from this pattern of concentration. Notably, Na increased by more than 250 times in the resulting DDGS, which was explained by the addition of NaOH for cleaning and pH regulation during ethanol production. Sulfur also increased by an average of 6.4-fold due to the addition of sulfuric acid for pH adjustment. Finally, Liu and Han (2011) reported a different pattern of P content during different stages of the dry-grind ethanol production process. In particular, the P content in DDGS increased by around three times the amount of that in corn. However, P in DDGS also differed in its nature relative to that in corn; most P in corn was in the form of phytate (~90%), while most in P in DDGS was in the form of non-

phytate P (55%) due to the effect of phytase produced by the yeast during the fermentation cornstarch into ethanol.

1.3.6. Vitamins Content in Corn DDGS

Corn is considered as a good source of vitamin E and carotenoids (pro-vitamin A compounds), which have antioxidant activity and have potential health benefits (Shin et al., 2018). Hence, corn products such as DDGS are expected to have greater content of these vitamins. There is growing interest in the value of these components in DDGS for animal feeding. Recently, the vitamin composition of corn and corn DDGS was revised (Shin et al., 2018; Chen et al., 2019). In general, the vitamin content in corn DDGS is about three times that of corn. The antioxidant capacity and potential applications of these and other phytochemicals present in corn DDGS are currently being investigated (Shurson, 2017).

1.4. CONSIDERATIONS FOR FEEDING DDGS TO MONOGASTRICS

1.4.1. Energy Value

1.4.1.1. Dietary Energy and Energy Systems. Feed components that generate energy are carbohydrates, proteins, and lipids. These components release energy by partial or complete oxidation following their digestion and absorption in the organism (Velayudhan et al., 2015). The form and availability of these fractions in the feed would influence the energetic value of the feed, as well as the ability of the animal to utilize them. For instance, fat has a high gross energy value and is highly digestible, whereas starch that is enzymatically hydrolyzed to glucose in the small intestine is more efficiently utilized as source of energy for swine than starch that escape enzymatic hydrolysis in the small intestine (i.e. resistant starch), but is fermented in the large intestine to yield volatile fatty

acids (**VFA**; Giuberti et al., 2014). Carbohydrates in form of fiber are poorly digested in the small intestine because pigs lack the enzymatic capacity to hydrolyze them; they contribute energy to pigs mainly via fermentation into VFA, and hence they are less efficient source of energy than fat and starch that is digested in the small intestine (Noblet and van Milgen, 2004).

Energy value of feedstuffs for swine is often evaluated on the basis of digestible energy (**DE**, which is gross energy content minus energy output in feces i.e., digestibility), Metabolizable energy (**ME**; DE minus energy output in urine and gases) content, or net energy content (**NE**; ME minus heat increment). The heat increment is the energy that is spent during feed ingestion, digestion, absorption and post-absorptive utilization of nutrients. Thus, NE is a more accurate estimator of the actual energy value of feedstuffs. The gross energy values for CP, fat, starch, and dietary fiber are estimated to be 5.5, 9.3, 4.2, and 4.4 kcal/g respectively, whereas the DE supply is 7.6 kcal/g for fat, 5.35 kcal/g for CP, 4.1 kJ/g for starch and 0.76 kcal/g for dietary fiber (Noblet and van Milgen, 2004). The efficiency of ME utilization for growing pigs of extract (**EE**), starch, digestible CP and digestible fiber is in average 90, 82, 58, and 58%, respectively (Noblet et al., 1999). Therefore, CP and dietary fiber have low NE value for growing pigs.

1.4.1.2. Energy Content of Corn DDGS. Corn DDGS has a greater gross energy value compared to that of corn (5,434 vs. 4,496 kcal/kg; NRC, 2012). Nonetheless, energy-contributing fractions differ considerably when the two are compared. Starch is the main source of energy in corn, whereas protein and fiber are the major sources of energy in DDGS. Consequently, DDGS has a higher heat increment and hence lower NE value than corn; the ratio NE to GE for DDGS was 0.46, whereas that for corn was 0.68 (Kerr and

Shurson, 2013). The ME value of corn DDGS and corn are on average similar (3,396 vs. 3,395 kcal/kg; NRC, 2012), whereas the NE value of DDGS is in general lower to that of corn (2,343 vs. 2,672 kcal/kg; NRC, 2012). Since the energy value of a feedstuff depends mainly on its composition of energy-yielding components, the energy value of DDGS varies depending on the composition of energy-yielding components in it. However, fat and fiber content are the major determinants of energy value of DDGS for pigs. For instance, NE:GE values for DDGS varied from 0.42 to 0.46 due to variation in its fat content (Kerr et al. (2013). Hence, equations for prediction of energy values in corn DDGS have been developed based on the chemical composition, primarily the EE and fiber contents (Li et al., 2015). As previously mentioned, the NSP content in corn DDGS is high compared to that of corn. The NSP cannot be digested by endogenous enzymes in monogastric animals; however, they can be fermented by some microorganisms of the gastrointestinal tract (**GIT**) yielding VFA and CO_2. These VFA produced during the fermentation of NSP can be used as source of energy in the host animals, and provide between 5 to 28% of the maintenance energy requirement, but still the loss of energy in forms of methane, hydrogen and heat decrease the efficiency of energy utilization (Kerr and Shurson, 2013). The efficiency of utilization of VFA as source of energy for maintenance and growth is on average 15% lower that of glucose, therefore the net energy supply from fermented carbohydrates has been estimated to be 75% that of enzymatically digested starch (Rérat, 1978; Bakker et al., 1998). Also, NSP as part of dietary fiber[3](**DF**) are traditionally considered as anti-nutritional factors in feed ingredients (Knudsen, 2014).

[3] There is still debate on the proper definition of dietary fiber, particularly on the actual constituents of what can be defined as fibers and therefore there is difficulty on the development of analytical measurements, the most recent format (AOAC Standard Method 2017.16.) includes “classical fibers” (cellulose, β-glucans, arabinoxylans, pectin, etc.), resistant starch and Non-digestible Oligosaccharides.

High NSP levels in DDGS can affect the digestibility of other nutrients due to an encapsulation effect, which reduces the accessibility of digestive enzymes to otherwise digestible components such as starch, protein and fat (Bedford, 2018). Furthermore, large amounts of NSP increase the weight of the GIT organs, particularly the hindgut, which in turn increases the energy requirements for maintenance at expense of skeletal tissue deposition (Bakker et al., 1998). Finally, NSP can negatively affect satiety and digesta transit time (Wenk, 2001).

1.4.2. Dietary Fiber and NSP Levels in Corn DDGS

The DF is composed primarily of NSP and lignin. As mentioned before, DF content in DDGS is high and has a negative impact on the NE value of the DDGS. Additionally, structural NSP might encapsulate other nutrients, thereby limiting digestibility of the nutrients. Thus, DF can limit inclusion of corn DDGS in swine diets. According to the NRC (2012) and Stein and Shurson (2009), corn DDGS could be included in diets for all production stages of market pigs, starting with two to three weeks post-weaning, at up to 30% DDGS, whereas in lactating and gestating sows, it can be included at 30 to 50% without negatively impacting the performance of swine. This is because older animals have a greater capacity to utilize fiber as energy source (Noblet and van Milgen, 2004). Nevertheless, the concentration in the diet of NSP can positively impact intestinal physiology by its physical presence in the intestinal lumen (Bach Knudsen et al., 2012). For instance, prebiotics which are undigested dietary carbohydrates that are fermented by colonic bacteria to yield short chain fatty acids as end products (Hutkins et al., 2016), are considered to have a beneficial effect on the host because they selectively stimulate the growth and/or activity of beneficial bacteria in the hindgut (Cummings and Stephen, 2007). This rationale has led a recent trend on research with the aim of determining the effects of

DF and NSP on nutrition and gut health of pigs (Lindberg, 2014). Prebiotic carbohydrates have been shown to modulate hindgut microbiome and promote gut-health (Jha and Berrocoso, 2015). Therefore, part of the DF could be considered as a useful resource, particularly during periods such as the post-weaning stage (Van Hees et al., 2019). Monteagudo-Mera et al. (2018), evaluated the prebiotic potential of arabinoxylans extracted from wheat distillers' dried grains with solubles, and successfully observed prebiotic activity of xylo-oligosaccharides and arabinoxylan oligosaccharides. For instance, they detected a lower growth of *Bifidobacterium* and higher propionate production for xylo-oligosaccharides and arabinoxylan oligosaccharides than for the classic prebiotics such as fructo-oligosaccharides. Furthermore, the rate of fermentation was lower for xylo-oligosaccharides and arabinoxylan oligosaccharides than fructo-oligosaccharides, and this can be important because this relatively slow fermentation would allow the oligosaccharides to reach the distal part of the hindgut where they can positively impact gut health (Monteagudo-Mera et al., 2018).

The use of diets with high levels of DF has been reported to have an important role on promoting satiety, thereby improving the welfare of sows that are subjected to feed restriction during pregnancy (Jarrett and Ashworth, 2018). Li et al. (2013) reported improvements in behavior and welfare of gestating sows fed diet with DDGS at 40%; however, these positive effects were only observed in sows that were individually housed in stalls and not in those that were group housed. In addition, some studies have reported that the use of DDGS in sow diets can induce oxidative stress, which can in turn, reduce birth and weaning weights of piglets (Wei et al., 2019). Thus, further research is needed to

explore efficient and precise ways of utilization of NSP in swine diets at different stages of production.

1.4.3. Protein Quality and Amino Acid Digestibility of Corn DDGS

Another important aspect of the use of DDGS in pig diets is its AA profile. Corn grain is low in Lys and Trp, and therefore corn DDGS has also moderate levels of Lys and Trp (NRC, 2012). Also, DDGS is subjected to heat and hence Maillard reactions may occur during its production. Maillard reactions occur when an amino group of AA reacts with a reducing sugar (e.g., fructose and glucose), resulting in reduction of the biological availability of the AA. However, AA that are affected by Maillard reactions are partially recovered during chemical analysis of feedstuffs by wet chemistry, leading to overestimation of the AA content (Almeida et al., 2013; Teodorowicz et al., 2018). Lys is the AA that is most affected by Maillard reactions, and therefore Lys availability is often a decisive characteristic when evaluating the quality of corn DDGS (Almeida et al., 2013). The Lys content is low in corn (0.25%; NRC (2012), while increased level is expected in corn DDGS (0.77%; NRC, 2012); however, the standardized ileal digestibility (**SID**) of Lys is lower in corn DDGS than in corn grain (61% SID Lys vs 74% SID Lys, respectively; NRC, 2012).

As previously mentioned, the occurrence of Maillard reactions is mediated by heat, and therefore is associated with temperature and the duration of heating of proteins in the presence of reducing compounds (Teodorowicz et al., 2018). The DDGS is highly susceptible to Maillard reactions and research has been conducted in order to better determine the availability of AA in corn DDGS (Fontaine et al., 2007; Pahm et al., 2008; Almeida et al., 2013). For instance, Zeng et al. (2017) and Almeida et al. (2013) have developed equations that predict the SID of AA of corn DDGS.

1.5. TECHNOLOGIES FOR IMPROVEMENT OF CORN DDGS UTILIZATION

As previously discussed, the high content of NSP and low quality of protein in DDGS limits its inclusion in monogastric diets. Therefore, research has been conducted in order to evaluate technologies that can result in a better utilization of corn DDGS as source of energy and protein in monogastric diets. Feeds and feedstuffs can be processed to increase their digestibility and reduce concentrations of antinutritional factors (Jansman, 2016). Several processing technologies that can potentially improve the nutritive value of corn DDGS, principally by disrupting the cell wall structure (i.e., NSP), have been proposed (de Vries et al., 2012). In addition to feed processing technologies, pretreatment technologies commonly used in second generation ethanol production (aka lignocellulosic bioethanol production) might be incorporated into ethanol plants to enhance the nutritive value corn DDGS (Chatzifragkou et al., 2015). Processing and pretreatment technologies can be classified as physical, chemical, physicochemical or biological treatments. Table 1.7 and Table 1.8 present some of the studies reviewed for this section.

1.5.1. Physical Processing

1.5.1.1. Mechanical Processing. Mechanical processing is one of the major types of physical processing technologies that are used to improve nutritive or feeding value of feeds or feedstuffs. Mechanical processing involves application of mechanical forces to the feedstuffs with the objective of altering its physical or chemical properties. One of the most widely used method of mechanical processing in the animal feed industry is the particle size reduction, which is achieved by techniques such as milling.

The particle size of DDGS can varies from 0.07 mm to 2.75 mm in diameter and it is dependent of the characteristics of the parent corn grain and the conditions under which

the corn grain is converted into ethanol and DDGS (Liu and Rosentrater, 2016). For instance, particle size of corn DDGS was highly correlated (R^2 = 0.807) with parent corn grain particle size (Liu, 2009a). Also, agglomeration of non-fermented components (fat, protein, and residual sugars) during the production of DDGS from corn grain affects the particle size of the resulting DDGS (Bhadra et al., 2009). Particle size of DDGS can affect its digestibility because nutrient digestibility is partly dependent on particle size. In pigs, for example, a particle size close to 600 μm is preferred with regard to improving digestibility of nutrients and growth performance, and optimization of energy utilization for milling (Wondra et al., 1995). Table 1.7 presents the effects of different types of physical processing technologies on the digestibility of corn DDGS. Yáñez et al. (2011), determined the effect reducing the mean particle size of DDGS from 517 μm to 383 μm on nutrient digestibility of the DDGS-based diets; they observed that grinding DDGS increased dietary SID of CP, SID of Lys, apparent total tract digestibility (**ATTD**) of NDF, and ATTD of GE; and DE value by 2.2, 6.2, 5.1, and 1.3 percentage units; and by 0.06 Mcal/kg, respectively. Other mechanical processing technologies include sieving, aspiration and winnowing; these technologies are commonly used in a process known as fractionation. Fractionation involves physical separation of the feedstuff particles based on theirs mass density (Chatzifragkou et al., 2015). For DDGS, light fractions would consist primarily of fiber, whereas heavy fractions would mostly consist of oil and protein (Liu, 2009b; Cheng and Rosentrater, 2017). Fractionation of DDGS can therefore result in two different products; fiber enriched fraction that could be utilized for ruminant feeding, lignocellulosic ethanol or other fiber industries, and non-fiber enriched fraction that can be utilized for feeding monogastric animals such as swine and poultry. The non-fiber enriched

product of fractionation of corn DDGS is often known as high protein DGGS (**HP-DDGS**). Espinosa and Stein (2018), evaluated the nutritive value of HP-DDGS and observed that the fractionated DDGS had a greater SID of Leu, Lys, Met, Phe, and Glu; and ME and DE values than conventionally produced DDGS. Similarly, Adeola and Ragland (2016) observed greater SID of Lys, Thr and Trp for HP-DDGS than for conventional DDGS fed to pigs.

Based on results from the fore-mentioned studies, it is apparent that mechanical processing can improve digestibility of energy and AA of corn DDGS for pigs. Particle size reduction increases energy and nutrient digestibility through an increase in surface area of feedstuffs such as DDGS, leading to increased exposure of nutrients to digestive enzymes, whereas fractionation increases energy and nutrient digestibility of DDGS by generating a product that has low fiber content, but high content of non-fiber components of DDGS.

1.5.2. Thermal Processing

Numerous processing techniques involve the application of heat at different levels and thus can be classified as thermal treatments (Rojas and Stein, 2017). Thermal processing can be performed on feed or feedstuffs before, during or after manufacturing process. Some of the post-manufacturing technologies employed in the production of feed for monogastric animals includes pelleting, extrusion and autoclaving (Kiarie and Mills, 2019). Technologies that are applied at early stages of the manufacturing process are often refer to as pretreatment technologies. Pretreatment technologies that involve use of heat include steam explosion and extrusion.

The effects of thermal processing technologies on nutritive value of DDGS for pigs have been reported. Zhu et al. (2010), observed improved ATTD of DM, organic matter (**OM**), GE and CP due to pelleting diets of nursery pigs that contained 30% DDGS. Rojas et al.

(2016), evaluated the effects of pelleting and extrusion individually or in combination on nutrient digestibility of pigs diets containing 25% of corn DDGS, and observed improved apparent ileal digestibility (**AID**) of starch and most indispensable AA and the ATTD of GE due to extrusion and pelleting with respect of the same diet offered in a meal form, however in general they did not observed differences between pelleting, extrusion or combination of both. Likewise, Oryschak et al. (2010) reported increments in the digestibility of AA of corn DDGS diets fed to broilers due to extrusion. On the other hand, Almeida et al. (2013) observed decreased SID of CP and some AA of DDGS for pigs due to autoclaving the DDGS at 130°C for 10, 20, or 30 min. Therefore, heat processing technologies such as pelleting and extrusion can increase nutrient digestibility in diets containing DDGS, whereas autoclaving DDGS can reduce AA digestibility, likely due to heat damage of the protein (occurrence of Maillard reactions).

Recently, the pretreatment technologies (including acid, alkali and hot water) at early stages of production of DDGS have been suggested as methods to improve nutritive value of DDGS for monogastric animals such as pigs and poultry (Zangaro et al., 2018). These technologies have been researched as methods for increasing release of sugars from lignocellulosic mass for production of ethanol (a.k.a. second generation bioethanol production technologies); the intention of the pretreatment technologies is to disrupt structure of fiber that is recalcitrant to enzymatic degradation or fermentation, thereby increasing the availability of fiber in the biomass for digestion (Amin et al., 2017). Nevertheless, it is worth mentioning that these technologies often involve use of extreme forces that might result in the damage of valuable nutrients in DDGS if applied to the latter. Therefore, these methods should be adapted, but optimized for pretreatment of corn DDGS.

Steam explosion is a technology that is used for the pretreatment of lignocellulosic mass. Steam explosion involves use of steam heating and shearing forces to break down structural components of the biomass material. Subjection of DDGS to steam explosion resulted in reduced dietary fiber level in the same feedstuff (Bryant et al., 2013; Iram et al., 2019). However, the effects of steam explosion on nutritive value of DDGS for monogastric animals have not been reported.

Some heat processing technologies can positively affect digestibility of nutrients in corn DDGS for pigs and poultry. However, in some cases heat processing technologies might negatively affect nutritive value of DDGS for pigs because they can reduce AA bioavailability due to Millard reactions. Furthermore, although results from *in vitro* studies indicate that heat pretreatment of WS improves its digestibility (Zangaro et al., 2018), research is needed to investigate effects of WS pretreatment and integration of this pretreatment technology into ethanol plants on nutritive value pretreated WS-derived DDGS for swine and poultry.

1.5.3. Chemical Processing

Chemical processing technologies involves use of chemicals such as acids, alkali solutions, organic solvents, and ionic liquids to disrupt cell wall structures of fibrous materials. Chemical pretreatment technologies have been used to increase availability of carbohydrates in lignocellulosic biomass for ethanol production (Agbor et al., 2011; Amin et al., 2017).

Pretreatment of lignocellulosic biomass with alkaline compounds results in disruption of bonds between lignin and cell wall carbohydrates, breakdown of lignin, increase in internal surface area by swelling, reduction in the polymerization degree of NSP, and reduction in crystallinity of cellulose (Amin et al., 2017). Alkalis that have been previously used for

pretreatment include sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide, and aqueous ammonia (Sindhu et al., 2015).

Alkali pretreatment with different compounds have been proven to be an effective technique for pretreatment of lignocellulosic biomass (Amin et al., 2017). Kim and Lee (2006) used hot-water and aqueous ammonia to pretreat corn stover for ethanol production and observed hydrolyzation of 92 to 95% of xylan fraction, removal of 75 to 81% of lignin, and the 90 to 96% of cellulose. Zaini et al. (2019), used NaOH pretreatment aiming to increase the enzymatic digestibility of cellulose of wheat DDGS for ethanol production, they found the optimum conditions of pretreatment with 5% NaOH at 121°C with yield of 88% of fermentable sugars.

Alkali treatment have been applied to cereal grains such as barley for ruminant animals with the goal of increasing digestibility of fibers and starch, but the results were inconsistent (Barnes and Ørskov, 1981; Campling, 1991; Naseroleslami et al., 2018). Studies with chickens have shown reduced AA digestibility in feather meal and sunflower meal as a result of alkali pretreatments (Provansal et al., 1975; Papadopoulos, 1989). In pigs, alkali pretreatment of barley had negative effects on nutrient digestibility when the barley was included in diets (Pringle et al., 1983), whereas alkali pretreatment of soybean meal (**SBM**) did not affect growth performance of weaning pigs (Wilson and Leibholz, 1981). The effects of ammonia pretreatment on nutritive value of DDGS fed to pigs or poultry have not been reported. Nevertheless, Zangaro et al. (2018) observed that pretreatment of corn WS with ammonia at high temperature and pressure increased *in vitro*

digestibility of DM by 15% without significant effect on Lys to CP ratio[4] in the WS (3.51 vs. 2.67 %).

Alkali pretreatments offer the advantage of low temperature operation compared to other chemical treatments, however, long residence time is needed followed by neutralization of the generated slurry in order to remove lignin and other inhibitors (e.g. phenolic acids, aldehydes, furfural and salts) of enzymes (Chatzifragkou et al., 2015). Furthermore, alkali pretreatment is not recommended for proteinous feedstuffs because it leads to racemization (conversion of an optically active compound into an optically inactive) of AA, which can negatively affect the bioavailability of AA (Liardon and Ledermann, 1986). For instance, Pringle et al. (1983) observed a reduction ATTD of CP (44.5 vs 65.1%) on pigs fed alkali treated barley. Therefore, alkali pretreatment technology can be used to degrade and de-lignify DDGS fiber. However, neutralization of the alkali pretreated DDGS slurry is needed. Moreover, although digestibility can be enhanced because of increased fiber solubilization, the bioavailability of AA in corn DDGS might be affected by alkali pretreatment.

Dilute acid pretreatment is the most used technology for pretreatment of lignocellulosic material and other tough feedstuffs such as feather meal (Papadopoulos, 1989; Agbor et al., 2011). The principle behind the use of acid pretreatments of lignocellulosic biomass is that hydronium ions which, originate from the acid catalyst cause breakdown of the long cellulose and hemicellulose chains into short fragments and simple sugars (Lloyd and Wyman, 2005). The acids that have been used for pretreatment include sulfuric acid, nitric acid and hydrochloric acid. Of these acids, sulfuric acid is the most widely used acid for

[4] The ratio of Lys to CP in a feedstuff is an indicator heat damage of AA in the feedstuff (Almeida et al., 2013)

the pretreatment (Agbor et al., 2011). The effects of dilute acid pretreatment of corn DDGS on the composition of the latter have been reported; however, the purpose of the pretreatment was to generate simple sugars for subsequent chemical or biological conversion into biofuel, solvents or value-added products, not specifically for animal feeding (Noureddini and Byun, 2010; Xu and Hanna, 2010; Chatzifragkou et al., 2015; Mikulski and Kłosowski, 2018; Iram et al., 2019). Noureddini and Byun (2010), pretreated corn DDGS with dilute sulfuric acid at loadings rates that ranged from 5 to 20 wt.% at 5% intervals, at acid concentrations that ranged from 0.5 to1.5 vol.% at 0.5% intervals, and at temperature of 120 or 140 °C; and observed conversion of the DDGS's NSP into simple sugars due to the pretreatment. In their study, the maximum concentration of simple sugars (xylose and glucose monomers) due to the pretreatment was 128 g/L and was achieved when the DDGS was pretreated with sulfuric acid at highest acid concentration and highest temperature. Recently, Iram et al. (2019) determined the effects of pretreating corn DDGS with dilute sulfuric acid at various concentrations and solid loading rates in an effort to identify optimal conditions for pretreating corn DDGS with dilute sulfuric acid. The maximum yield of sugars due to the pretreatment was 0.382 g per gram of DDGS, and this was achieved at 5% sulfuric acid concentration and 30% solid loading rate. At these conditions, furfural and 5-hydroxymethyl furfural (**5-HMF**) were generated at 5.2 and 1.6 mg/g DDGS, respectively. Ezeji and Blaschek (2008), investigated the effect of incubating acid-pretreated corn DDGS with solventogenic clostridia on production of acetone, butanol and ethanol, and observed increased solventogenic clostridia growth and production of acetone, butanol and ethanol from the DDGS due to the solventogenic clostridia incubation. However, the growth of the solventogenic clostridia was inhibited by

enzyme inhibitory compounds such as p-coumaric acid, ferulic acid, and syringaldehyde that were generated due to acid pretreatment.

The use of dilute acids for the pretreatment of corn DDGS for the use in swine diets has been also reported in the literature, and might represent attractive methods of increasing the susceptibility of DDGS for enzymatic digestion (de Vries et al., 2013; Zangaro et al., 2018). de Vries et al. (2014), investigated the effects of acid extrusion on the degradability of corn DDGS, and reported that although acid extrusion seemed to facilitate faster degradation of NSP and shifted fermentation to more proximal GIT segments, the overall extent of NSP degradation was not affected by the acid extrusion. Tucker et al. (2004), pretreated DDGS with dilute acid for production of ethanol and protein-enriched coproduct (58 to 61% CP) and reported that the inclusion of the high-protein feedstuff in turkey diets did not negatively affect performance.

Some potential drawbacks of dilute acid pretreatment includes the generation of high levels of sugar degradation compounds such as furfural and 5-HMF as well as aromatic lignin degradation compounds, which can impede fermentation process if the biomass is not detoxified (Ezeji and Blaschek, 2008; Chatzifragkou et al., 2015). Also, some of these compounds might inhibit enzymatic activity in the small intestine (Martinez-Gonzalez et al., 2017). Notwithstanding, this drawback of dilute acid pretreatment of lignocellulosic material might represent a potential application in monogastric nutrition. This is because the inhibition of pathogenic bacteria (particularly in the post-weaning period) through different technologies have been evaluated as alternative to the use of in-feed antibiotics in swine (Suiryanrayna and Ramana, 2015). Future research would need to determine if the generation of inhibitory compounds might inhibit potentially harmful bacterial in the gut

of pigs fed pretreated corn DDGS and favor the growth of beneficial bacteria that can use the fiber from pretreated DDGS for the generation of volatile fatty acids that are utilize as source of energy by the animals. Consequently, diluted acid pretreatment technology could be used to enhance digestibility of corn DDGS fiber in monogastric animals. However, the generation of furans and lignin degradation products by the acid pretreatment is a concern. Research is needed to determine effects the effects of acid pretreated DDGS on nutrient digestibility, gut health and performance of monogastric animals such as pigs and poultry.

1.5.4. Physicochemical Processing

Physicochemical processing technologies involves a combination of chemical and physical processing. They include ammonia fiber explosion (**AFEX**) and liquid hot water treatment (**LHW**). Several chemical methods (e.g. ammonia pretreatment, acid pretreatment) often require the use of heat and will therefore not be addressed further in this section.

The AFEX procedure involves exposure of ammonia to substrate (lignocellulose mass or fibrous feedstuffs) at a given temperature and high pressure, which results in swelling and loss of crystallinity of cellulose, breakdown of bonds between lignin and NSP, and degradation of lignin (Agbor et al., 2011). However, hemicellulose polysaccharides are poorly disrupted during AFEX pretreatment (Chatzifragkou et al., 2015). In the case of DDGS, AFEX is performed under relatively mild conditions (temperatures below 90 °C and pressure range of between 200 and 400 psi) due to the relatively low lignin content of corn DDGS compared to other lignocellulosic materials (Chatzifragkou et al., 2015). Bals et al. (2006), observed complete conversion of cellulose to glucose due to AFEX pretreatment of dry and wet DDGS, whereas xylose yield after AFEX treatment was negligible; only small amounts of furfurals were generated by the pretreatment. The optimal conditions for AFEX pretreatment of dry and wet DDGS were 70° C and 0.8 kg

anhydrous NH3/kg dry biomass, and 80° C and 0.6 kg NH3/kg dry biomass, respectively. Urriola P et al. (2018), observed increased *in vitro* digestibility of GE and thereby increased estimated digestible energy content of corn DDGS by about 700 kcal/kg due to AFEX pretreatment at 300 psi and 100°C. Potential challenges of this method includes the cost of ammonia and environmental concerns due to generation of unpleasant odors and the fact that ammonia must be recycled after the pretreatment (Taherzadeh and Karimi, 2008; Chatzifragkou et al., 2015).

The LHW pretreatment involves exposing biomass to hot water under target pressures and temperatures over the boiling point, these processes are also known as autohydrolysis, hot compressed water or hydrothermolysis (Chatzifragkou et al., 2015). In general, these methods can result in nearly complete dissolution and hydrolysis of hemicellulose, partial dissolution of lignin or sometimes even overall dissolution of biomass including cellulose (Yan et al., 2016; Zhuang et al., 2016). Li et al. (2019), reported complete cellulose conversion to glucose as consequence of LHW pretreatment of corn DDGS at 160 °C and 20 min under high pressure conditions. Samala et al. (2012), observed conversion of more than 50% of the initial xylan content to xylo-oligosaccharides and trace amounts of inhibitors due to LHW pretreatment of corn DDGS fiber at 180 °C for 20 min. Information is lacking on the effects of incorporating LHW-pretreated DDGS in swine diets on nutrient utilization. Nevertheless, Zangaro et al. (2018) observed increased *in vitro* digestibility and enhanced fermentation of WS due to pretreatment of the latter with heat at 160 °C and 70 psi for 20 min. Since WS is a high moisture biomass (~90%) the pretreatment implemented in the study of Zangaro et al. (2018) may well be considered as a form of LHW. Formation of fermentation inhibitors can occur during LHW pretreatment as a consequence of

monosaccharide degradation into furfural from pentoses and 5-HMF from hexoses, nonetheless this can be controlled by keeping the pH between 4 and 7 (Taherzadeh and Karimi, 2008; Chatzifragkou et al., 2015).

The AFEX and LHW treatments are promising technologies for increasing value of corn DDGS by disruption of fiber components. However, the mechanisms might differ depending on the technology used. The AFEX can increase cellulose degradation but this pretreatment does not disrupt matrix structure of arabinoxylans, which constitute the highest proportion of total NSP in corn DDGS. Thus, AFEX might not be so effective in improving the nutritive value of DDGS for pigs; however, there is need to confirm this through research. On the other hand, LHW pretreatment mostly generates oligosaccharides under mild conditions, and hence low production of inhibitory compounds because the inhibitory compounds such as furfural and 5-HMF are derived from monosaccharides. Furthermore, the generated oligosaccharides might be readily fermentable in the hind gut of pigs to supply energy.

1.5.5. Biological Pretreatment

Biological pretreatment involves the use of microbes and/or enzymes for disruption of recalcitrant cell wall structures (Amin et al., 2017). Biological treatments are often considered as advantageous in comparison to other pretreatment methods, because: (1) they required less energy, (2) involve use of less amounts of harmful compounds such as alkalis or acids, and (3) do not generate compounds that inhibit fermentation or activity of digestive enzymes (Sindhu et al., 2016).

Kim et al. (2008), observed solubilization of 76% of cellulose in corn DDGS due to predigestion of the latter (at low solid loading rate; 5%, w/w) with cellulase and β-glucosidase enzymes for 72 h. Furthermore, pretreatment with AFEX and LHW of DDGS

and subsequent hydrolysis with enzymes mixtures resulted in nearly complete (98%) cellulose hydrolysis (Kim et al., 2008). Zangaro et al. (2018), observed increased *in vitro* digestibility of WS by 18% due to predigestion of the WS with a multienzyme product that contained NSPase, phytase and protease for 24h. Rho et al. (2018a), did not observe change in performance of growing pigs due to dietary inclusion of corn DDGS that had been steeped without or with exogenous feed enzymes (xylanase, β-glucanase, cellulase, and protease) for 24 h. However, feed efficiency of growing pigs fed DDGS-based diets was improved due to fermentation of the DDGS with a blend of β-glucanase and xylanases for 74h (Rho et al., 2018b). Some potential drawbacks with regard to the predigestion of fibrous feedstuffs with enzymes include reduced accessibility of enzymes to their target substrates (due to complex cell wall structure), inhibition of enzymatic activity by the end-products of digestion (negative feedback), unexpected enzyme activities, discordance in optimum conditions of different types of enzymes and the high cost of tailored designed enzyme cocktails for specific applications (Chatzifragkou et al., 2015).

The effects of pretreating lignocellulose mass with microorganisms have been reported. The most promising microorganisms for biological pretreatment are white-rot fungi that belong to class Basidiomycetes (Taniguchi et al., 2005). Taniguchi et al. (2005), reported reduction in lignin, cellulose and hemicellulose content of rice straw by of 25, 17 and 48%, respectively, due to pretreatment of the latter with *Pleurotus ostreatus* for 60 days. Shrestha et al. (2009), observed enhanced conversion of corn fiber into simple sugars and enrichment of the biomass residue with fungal protein due to solid-substrate fermentation of corn fiber with either white- or brown-rot fungi. Salvachúa et al. (2011), pretreated wheat straw with *Poria subvermispora* and *Irpex lacteus* for 21 d with goal of converting

cellulose into glucose for fermentation into alcohol and reported conversion of at least 66% of cellulose into glucose. The main limitation of pretreatment of lignocellulose mass with microorganisms is the long periods needed for the process (Taherzadeh and Karimi, 2008). Based on results from the fore-mentioned studies, it is apparent that biological pretreatment technologies represent a good option for improving nutritive value of DDGS for monogastric animals such as pigs. This is because they require low energy input, do not require the use of corrosive chemicals, and have less negative impact on environment. Furthermore, when fungal microorganisms are used the pretreatment, the pretreated feedstuff might have greater protein value for monogastric animals. However, long periods of time are needed for pretreatment of lignocellulose mass with microorganisms. Thus, enzymatic pretreatment might be more suitable technology for pretreatment of DDGS for monogastric animals.

1.6. CONCLUDING REMARKS

Corn DDGS is a feedstuff with remarkable nutrient characteristics for swine feeding. However, its high NSP levels and low protein quality represent a challenge for optimized utilization of this coproduct in swine diets. Because of the complex matrix structure of DDGS, several processing technologies that target specific components of interest in DDGS have been proposed (Table 1.7). The NSP solubilization by action of pretreatment technology might be an option that results in increased energy value of DDGS for pigs because of NSP conversion in simple sugars and readily fermentable oligosaccharides by the pretreatment, and of reduced antinutritional effects of NSP. Utilization of heat-pressure pretreatment technologies might represent an opportunity for enhancing nutritive value of corn DDGS because of their effectiveness in disrupting fiber components and the low generation of toxic compounds. Moreover, these technologies can be incorporated into the

ethanol plants to reduce the cost of pretreated DDGS. Enzymatic pretreatment technologies can also be effective with regard to enhancement of nutritive value of corn DDGS for pigs because they do not require the use of corrosive chemicals, the low generation of toxic compounds, and the fact that technologies can be combined with other types of pretreatment technologies for enhancement of nutritive value of corn DDGS for pigs. Nevertheless, most of the research related to pretreatment technologies has been on their use to convert fiber in lignocellulose mass into simple sugars for ethanol production. Furthermore, extreme conditions (very high temperature and pressure) that result in generation of compounds that can reduce digestibility and fermentation of feed in digestive tract of monogastric animals were used in the fore-mentioned studies. Therefore, there is a need to identify optimal conditions for pretreating DDGS for monogastric animals such as pigs and poultry.

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Tables and figures

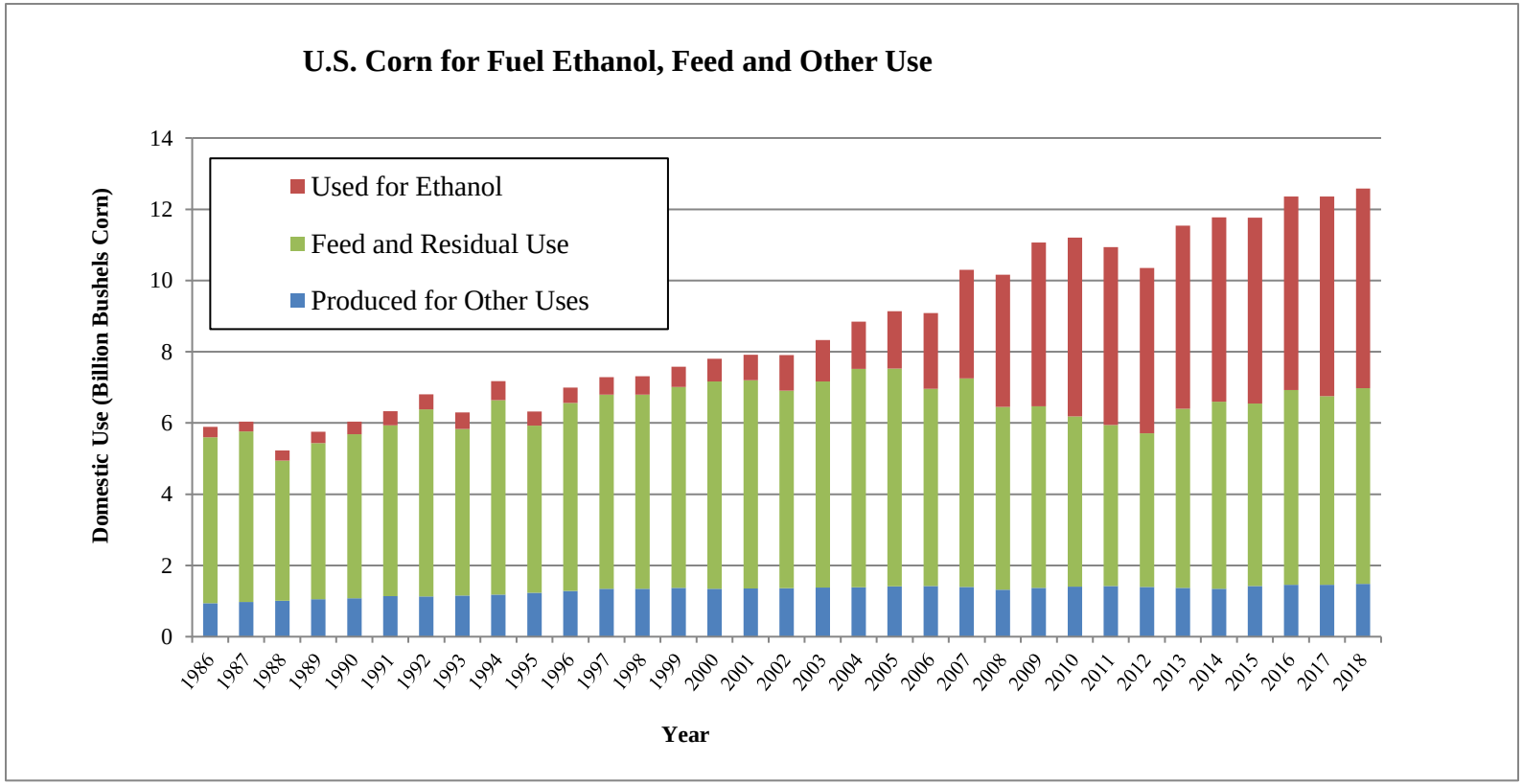

Figure 1.2. Historic U.S. total corn production & used (Million Bushels). The trend is generally increasing, used for ethanol has grown apparently at expense feed and residual use, which has slightly decreased, other uses includes human food and it has remained historically steady. Year 2012 show a depression due to a drought event (AFDC, 2019).

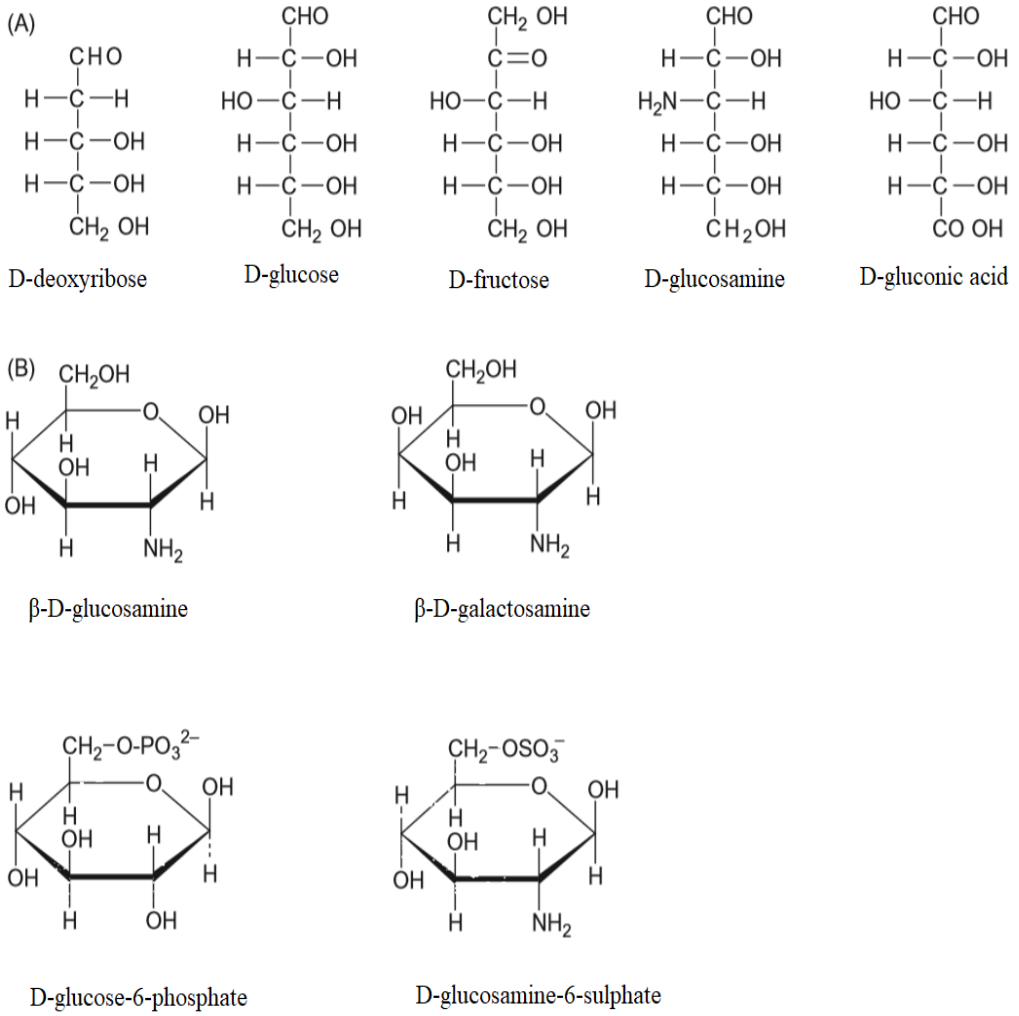

Figure 1.3. (A) Structural variation among sugars. (B) Sugars containing N, S, and P. Adapted from: Dilworth et al. (2017)

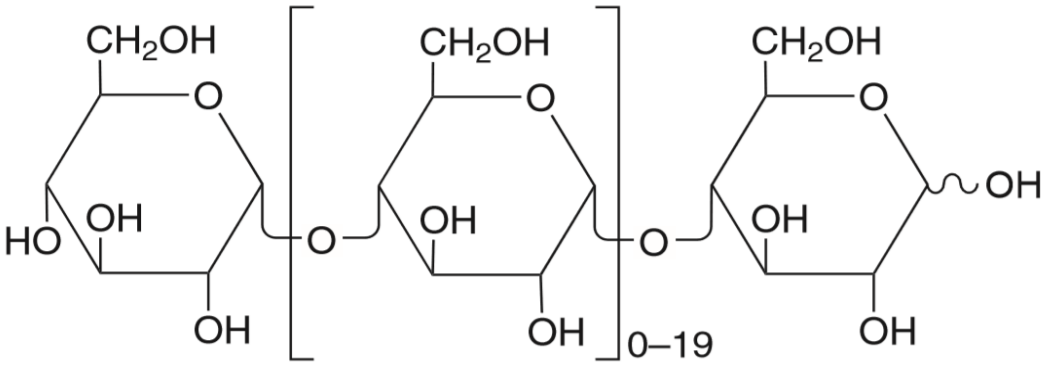

Figure 1.4. An example of a Malto-oligosaccharide (n= 0-19), molecules obtained from starch. Here each monosaccharide unit is an a-D-glucopyranosyl unit, and each is joined to the O4 position of the unit to the right of it by a glycosidic bond (From: (BeMiller, 2018).

Table 1.1 Starch content (DM basis) of corn and corresponding DDGS (Pedersen et al., 2014).

	Feedstuff	
Item	Corn	DDGS
Mean	72.3	5.1
Range	70.5-76.6	4.3-5.9
SD	11	5
CV	0.01	0.1
n	11	11

Table 1.2. Compositional profile of corn and corresponding DDGS (g/kg DM) (Extracted from: (Pedersen et al., 2014)

	Corn (N = 11)			Corn DDGS (N = 11)			
Item	Mean	Range	S.D.(CV)	Mean	Range	S.D.(CV)	Ratio[i]
Total NSP [ii]							
Total	79b	(67–91)	7(0.08)	325c	(313–337)	8(0.02)	4.1
Soluble	6b	(2–10)	3(0.39)	29c	(18–37)	6(0.19)	4.5
Cellulose	17b	(14–20)	2(0.12)	79c	(74–91)	5(0.06)	4.6
NCP							
Xylose							
Total	23b	(20–27)	2(0.09)	94c	(88–100)	3(0.04)	4
Soluble	1b	(0–1)	1(0.97)	5c	(1–8)	2(0.48)	7.6
Arabinose							
Total	18b	(15–20)	1(0.07)	69c	(65–72)	2(0.04)	3.8
Soluble	1b	(0–2)	1(0.63)	7c	(3–9)	2(0.25)	5.8
Glucose							
Total	7b	(6–8)	1(0.09)	27c	(22–29)	2(0.06)	3.7
Soluble	1b	(0–1)	1(0.97)	1b	(0–5)	2(1.35)	2.4
Mannose							
Total	2b	(2–3)	0(0.10)	17b	(14–18)	1(0.06)	7.5
Soluble	1b	(1–1)	0(0.14)	7c	(5–8)	1(0.13)	6.8
Galactose							
Total	6b	(4–7)	1(0.13)	20c	(18–21)	1(0.05)	3.6
Soluble	1b	(1–2)	0(0.38)	3c	(2–4)	1(0.17)	2.5
Uronic acids							
Total	5b	(4–6)	1(0.10)	19c	(18–20)	1(0.04)	3.5
Soluble	1b	(1–2)	0(0.19)	5c	(5–6)	0(0.08)	3.5
Klason lignin	10b	(7–15)	2(0.23)	38c	(28–47)	5(0.13)	3.9
A/X-ratio	0.77b	(0.74.0.79)	0.0(0.02)	0.73c	(0.71–0.74)	0.0(0.01)	
UA/X-ratio	0.23b	(0.22–0.24)	0.0(0.03)	0.20c	(0.19–0.21)	0.0(0.03)	
Cel./T-NSP ratio	0.21b	(0.20–0.23)	0.0(0.05)	0.24c	(0.20–0.23)	0.0(0.05)	

[i] Average corn DDGS-to-corn ratio.

[ii] A/X ratio, arabinose-to-xylose ratio; CV, coefficient of variation; NCP, non-cellulosic polysaccharides; NSP, non-starch polysaccharides; UA/X ratio, uronic acid-to-xylose ratio.

Table 1.3. Fiber profile of corn and eight sources of distillers dried grains with solubles (Espinosa et al., 2019).

	Feedstuffs											
Item, %[i]	Corn	A	B	C	D	E	G	H	I	Mean	SD	CV, %
NDF	7.38	36.29	33.89	34.10	31.64	27.90	27.15	28.75	28.59	31.04	3.41	11.00
ADF	2.75	16.33	14.51	12.28	14.69	16.20	11.87	11.39	10.84	13.51	2.19	16.20
SDF	1.87	0.33	3.83	3.63	2.89	3.12	2.52	1.35	2.03	2.46	1.18	48.10
IDF	1.30	39.31	37.11	38.31	36.52	33.68	33.67	34.93	35.23	36.10	2.08	5.80
TDF	3.17	39.64	40.95	41.94	39.41	36.80	36.20	36.28	37.26	38.56	2.22	5.80

[i] NDF: Neutral detergent fiber, ADF: Acid detergent fiber, SDF: Soluble dietary fiber, IDF: Insoluble dietary fiber, TDF: Total dietary fiber

Table 1.4. Changes in amino acid Composition (Relative % of an Individual AA vs Total AA in Each Sample) during the Dry Grind Ethanol Process from Corn (Han and Liu, 2010).

	Resource											
Item	Ground corn	Cooked slurry	Liquefied mass	Saccharified mass	Fermented mass	Whole stillage	Thin stillage	Distiller solubles	Distiller grains	WDGS	DDGS	Yeast
Essential												
Arg	3.55 ± 0.43	3.96	3.4	4.05	4.51	4.98	6.11	5.09	4.42	5	4.68 ± 0.13	5.15 ± 0.15
His	3.13 ± 0.17	3.85	3.09	4.05	2.86	3.57	3.84	3.13	3.28	2.98	3.59 ± 0.06	2.81 ± 0.07
Ile	3.61 ± 0.35	3.08	3.5	3.04	3.71	3.15	2.84	3.56	3.25	3.85	3.25 ± 0.02	4.46 ± 0.00
Leu	12.23 ± 0.25	11	11.32	11.49	11.36	11.23	7.95	8.4	13.07	11.38	12.16 ± 0.06	7.67 ± 0.18
Lys	3.19 ± 0.09	3.41	3.19	3.15	3.99	4.13	4.76	5.03	3.84	4.22	3.87 ± 0.05	8.31 ± 0.04
Met	3.30 ± 0.74	4.84	3.29	3.83	2.38	2.82	3.55	2.88	2.25	1.93	2.68 ± 0.10	2.14 ± 0.08
Phe	6.49 ± 0.32	5.28	6.79	5.97	5.4	4.98	4.47	5.03	5.22	5.05	4.90 ± 0.18	4.66 ± 0.20
Thr	3.96 ± 0.03	4.29	3.81	4.28	4.07	4.27	4.55	4.29	4.25	4.22	4.23 ± 0.01	5.95 ± 0.00
Val	7.50 ± 0.12	7.37	7.51	7.21	5.56	5.35	6.11	6.38	5.12	5.64	5.22 ± 0.00	5.43 ± 0.07
Non-essential												
Ala	6.56 ± 0.26	7.04	6.58	6.76	7.25	7.42	7.6	7.48	7.26	7.52	7.42 ± 0.01	5.79 ± 0.00
Asp	5.91 ± 0.33	6.71	6.38	6.64	6.97	7.19	7.46	7.36	6.95	7.16	7.06 ± 0.00	10.83 ± 0.06
Cys	2.95 ± 0.42	3.63	2.98	3.6	2.09	2.25	2.91	2.39	2.11	1.93	2.06 ± 0.02	1.38 ± 0.12
Glu	17.73 ± 0.66	17.93	17.7	17.79	18.73	19.4	19.03	18.15	19.53	18.85	19.52 ± 0.09	20.47 ± 0.08
Gly	3.43 ± 0.26	3.63	3.6	3.83	4.23	4.42	5.47	5.33	3.77	4.54	4.11 ± 0.05	4.66 ± 0.01
Pro	6.67 ± 0.23	4.84	7.3	5.52	7.81	6.39	4.97	6.74	7.16	7.66	6.90 ± 0.00	2.18 ± 0.25
Ser	5.02 ± 0.05	5.28	4.73	5.41	4.99	5.17	5.11	4.78	5.25	5.09	5.12 ± 0.03	5.52 ± 0.14
Tyr	4.82 ± 1.54	4.07	4.94	3.6	4.11	3.19	3.34	3.92	3.28	2.98	3.23 ± 0.00	2.60 ± 0.46

Table 1.5. Amino acid composition (g/kg) of corn Distillers' Dried Grains with Solubles (Olukosi and Adebiyi, 2013).

Item	Content, g/kg					
	n	Max	Min	Mean	SD	CV (%)
Essential						
Arg	26	14.6	10.6	12.2	0.978	7.99
His	24	9.1	6.5	7.37	0.695	9.43
Ile	27	12.5	9.6	10.7	0.723	6.73
Leu	24	36.2	28.9	32.1	2.1	6.57
Lys	28	11.1	6.2	9.01	1.18	13.1
Met	28	7.2	4.4	5.24	0.628	12
Phe	24	15.1	10.9	12.9	1.23	9.59
Thr	28	11.6	9.3	10.3	0.668	6.46
Trp	27	2.6	1.6	2.16	0.222	10.3
Val	26	16.1	13	14.2	0.949	6.7
Non-essential						
Ala	21	21	15.6	18.3	1.39	7.61
Asp	21	19.7	14.9	17.3	1.32	7.62
Cys	26	7	4.1	5.14	0.571	11.1
Glu	21	54.8	29.3	36.1	6.17	17.1
Gly	21	12.4	9.5	10.8	0.732	6.81
Pro	21	22.1	16.6	19.3	1.67	8.68
Ser	22	14.5	10.1	11.7	1.07	9.13
Tyr	22	12	9.1	10.1	0.731	7.22

Table 1.6. Amino acid composition of eight sources of distillers dried grains with solubles (Espinosa et al., 2019).

	Source of distillers dried grains with solubles										
Item, %	A	B	C	D	E	G	H	I	Mean	SD	CV, %
CP	29.40	28.60	29.55	28.93	28.42	29.82	27.87	27.14	28.72	0.90	3.2
Essential											
Arg	1.24	1.21	1.28	1.24	1.24	1.27	1.15	1.22	1.23	0.04	3.2
His	0.72	0.74	0.74	0.76	0.72	0.77	0.71	0.76	0.74	0.02	3.1
Ile	1.1	1.12	1.11	1.12	1.03	1.09	1.04	1.12	1.09	0.04	3.4
Leu	2.98	3.22	3.08	3.2	2.88	3.2	2.87	2.99	3.05	0.14	4.7
Lys	1.02	0.98	1.05	1	0.98	0.99	1.01	1	1	0.02	2.4
Met	0.52	0.53	0.53	0.47	0.52	0.53	0.5	0.51	0.52	0.02	3.9
Phe	1.26	1.34	1.29	1.35	1.24	1.34	1.22	1.27	1.29	0.05	3.7
Thr	1.11	1.09	1.12	1.09	1.03	1.14	1.04	1	1.08	0.05	4.6
Trp	0.2	0.21	0.21	0.2	0.19	0.19	0.17	0.19	0.2	0.01	6.1
Val	1.5	1.5	1.53	1.49	1.41	1.46	1.4	1.41	1.46	0.05	3.5
Total	11.65	11.94	11.96	11.92	11.24	11.99	11.12	11.48	11.66	0.35	3
Non-essential											
Ala	1.81	1.96	1.91	1.84	1.78	1.98	1.86	1.9	1.88	0.07	3.7
Asp	1.77	1.81	1.81	1.79	1.74	1.81	1.76	1.81	1.79	0.03	1.6
Cys	0.49	0.51	0.5	0.53	0.56	0.54	0.5	0.59	0.53	0.03	6.1
Glu	3.28	3.97	3.59	3.81	3.87	3.89	3.95	4.07	3.8	0.26	6.7
Gly	1.15	1.12	1.16	1.11	1.13	1.13	1.07	1.1	1.12	0.03	2.6
Ser	1.2	1.25	1.25	1.21	1.13	1.33	1.15	1.07	1.2	0.08	6.6
Tyr	0.93	0.96	0.91	0.96	0.88	1.02	0.86	0.94	0.93	0.05	5.4
Total	10.63	11.59	11.13	11.25	11.09	11.7	11.16	11.49	11.26	0.34	3
All AA	22.29	23.52	23.09	23.17	22.34	23.69	22.28	22.97	22.92	0.56	2.4

Table 1.7. Effects of processing methods on digestibility of corn DDGS fed to pigs.

	Adeola and Ragland (2016)			Espinosa and Stein (2018)			Yáñez et al. (2011)			Rojas et al., 2016			Oryschak et al. (2010)		
	Fractionation			Fractionation			Grinding			Extrusion			Extrusion		
Item	V[5]	% DIF[6]	S[7]	V	% DIF	S	V	% DIF	S	V	% DIF	S	V	% DIF	S
CP, % SID				78.70	2.21	NS	87.20	2.59	S	72.50	7.46	S	71.24	11.57	S
Indispensable AA, % SID															
Arg	96	3.11	NS	87.40	-2.24	NS	89.80	2.86	S	91.57	3.74	S	14.32	0.14	S
His	91.7	2.92	NS	82.20	3.27	NS	84.80	2.05	S	85.78	3.21	S	11.96	0.12	S
Ile	92.4	3.13	NS	81.90	4.46	S	87.70	0.92	NS	84.32	7.07	S	14.68	0.15	S
Leu	94.1	2.28	NS	89.20	5.31	S	88.10	1.61	S	87.14	5.98	S	8.77	0.09	S
Lys	85.8	7.38	NS	76.20	9.64	S	70.50	9.64	S	81.77	4.83	S	34.14	0.34	S
Met	94.6	1.94	NS	87.20	4.68	S	86.10	1.18	NS	87.70	5.31	S	10.10	0.10	S
Phe	93.6	3.20	NS	85.50	4.52	S	89.90	1.58	S	87.25	7.40	S	9.80	0.10	S
Thr	93.5	5.77	S	75.00	1.76	NS	80.00	2.43	NS	75.74	6.89	S	14.98	0.15	S
Trp	97	4.75	S	79.50	-0.87	NS	91.20	-0.87	NS	83.20	6.60	S	5.74	0.06	S
Val	92.3	4.41	NS	81.10	4.51	S	84.30	1.44	NS	80.46	6.37	S	13.94	0.14	S
Dispensable AA, %SID															
Ala	94.1	2.95	NS	84.50	3.30	NS	81.10	2.92	S	80.31	7.40	S			
Asp	91.2	6.05	NS	72.90	1.53	NS	70.10	5.41	S	80.29	4.76	S			
Cys	90.8	4.01	NS	74.90	2.32	NS	82.20	1.73	NS	67.92	1.81	NS			
Glu[8]	93.7	2.29	NS	87.50	5.29	S	91.90	0.77	S	85.42	6.52	S			
Gly	99.2	9.37	S	71.20	-7.89	NS	85.20	5.71	S	62.74	12.74	S			
Pro	103.9	2.06	NS		0.00		98.00	7.34	NS	71.64	10.23	NS			
Ser	95.1	4.51	S	81.80	1.11	NS	83.30	1.83	NS	82.93	4.90	S			
Tyr	94.2	2.50	NS	86.50	3.10	S	88.60	1.72	S	87.93	5.14	S			
Energy															
GE, %ATTD				77.79	0.03		84.90	0.01	S						
GE, %AID							76.40	0.03	S	85.54	0.84	NS	54.49	0.18	S
DE, kcal/kg				4424	0.07		3.50	0.01	NS						

[5] Reported value of the item
[6] Difference from control treatment
[7] Significance: S: Significant at $P < 0.05$, NS: No significant difference

	Adeola and Ragland (2016)			Espinosa and Stein (2018)			Yáñez et al. (2011)			Rojas et al., 2016			Oryschak et al. (2010)		
	Fractionation			Fractionation			Grinding			Extrusion			Extrusion		
Item	V[5]	% DIF[6]	S[7]	V	% DIF	S	V	% DIF	S	V	% DIF	S	V	% DIF	S
ME, kcal/kg				4275	0.07										
NE, kcal/kg															
Minerals															
P, %AID							48.90	-0.06	NS						
Ca, %AID							49.40	-0.18	S						

Table 1.8 Reported composition of corn DDGS treated or processed under different technologies.

Item,	Adeola and Ragland, 2016	Espinosa and Stein, 2018	Yáñez et al., 2011	Rojas et al., 2016	Oryschak et al. 2010	Zangaro et al, 2018				de Vries et al., 2013	Iram et al., 2019	Bals et al. 2006	Urriola P et al. 2018	Rho et al. 2018
	Fractionation	Fractionation	Grinding	Extrusion	Extrusion	Heat	CA[9]	H_2S0_4	NH_3	Maleic acid	DA, AFEX[10]	AFEX	AFEX	Enzymatic
CP g/kg	54.78	34.26	33.92	30.74	38.7	33.34	33.34	31.15	38.84	31	30.99	49.4	42.9	28.19
EE, g/kg	3.07	7.89	8.75	15.12	9.6	11.81	12.35	13.56	11.86		9.09	10.4	10.55	9.95
NDF g/kg	31.77	42.7	43.54	29.6	48.09						32.24	13.8	20.85	33.58
ADF g/kg	18.22	20.01	12.14	11.91	11.66						12.71		10.9	10.88
Ca, g/kg	0.06		0.12	0.06	0.16									
Total P, g/kg	0.28		0.93	0.76	1.02									
Ash, %		4.36	5.36	4.46	5.37						4.95	3.4	5.5	7.05
Starch		4.3	2.19		1.36					3				
TDF		40.69	28.56											
IDF		39.53	24.29											
SDF		1.16	4.27											
NSP						23.34	12.38	18.78	18.07	28				
Arabinose						2.85	0.44	3.92	3.22					

[9]CA: Citric Acid pretreatment
[10] DA: Diluted acid pretreatment, AFEX: Ammonia fiber expansion.

Item,	Adeola and Ragland, 2016	Espinosa and Stein, 2018	Yáñez et al., 2011	Rojas et al., 2016	Oryschak et al. 2010	Zangaro et al, 2018				de Vries et al., 2013	Iram et al., 2019	Bals et al. 2006	Urriola P et al. 2018	Rho et al. 2018
	Fractionation	Fractionation	Grinding	Extrusion	Extrusion	Heat	CA[9]	H_2S0_4	NH_3	Maleic acid	DA, AFEX[10]	AFEX	AFEX	Enzymatic
Xylose						6.76	2.73	5.12	5.21					
Mannose						1.36	1.27	1.24	1.38					
Galactose						1.14	0.54	0.96	0.98					
Glucose						7.65	6.61	5.46	6.21					
Uronic acid						1.24	0.8	1.13	1.07					
Lignin													1.8	
Indispensable AA, %														
Arg	1.77	1.57	1.67	1.53	1.72								1.25	
His	1.56	0.92	0.86	1.04	0.86								0.83	
Ile	2.19	1.4	1.43	1.17	1.51								1.25	
Leu	7.69	3.92	3.02	3.49	3.11								3.45	
Lys	1.21	1.24	1.14	1.15	1.04	1.09	0.9	0.87	1.04				0.84	
Met	1.12	0.63	0.59	0.6	0.64								0.57	
Phe	2.81	1.93	2.04	1.49	1.77								1.5	
Thr	1.91	1.33	1.26	1.19	1.22								1.1	
Trp	0.28	0.3	0.3	0.22	0.37								0.13	

Item,	Adeola and Ragland, 2016	Espinosa and Stein, 2018	Yáñez et al., 2011	Rojas et al., 2016	Oryschak et al. 2010	Zangaro et al, 2018				de Vries et al., 2013	Iram et al., 2019	Bals et al. 2006	Urriola P et al. 2018	Rho et al. 2018
	Fractionation	Fractionation	Grinding	Extrusion	Extrusion	Heat	CA[9]	H_2S0_4	NH_3	Maleic acid	DA, AFEX[10]	AFEX	AFEX	Enzymatic
Val	2.85	1.91	1.64	1.55	1.88								1.6	
Dispensable AA, %														
Ala	4.45	2.35	1.67	2.17	1.76								2.1	
Asp	2.98	2.19	1.97	1.92	2.04								1.95	
Cys	0.92	0.66	0.7	0.6	0.74								0.23	
Glu	7.33	4.43	7.23	4.07	8.49								4.2	
Gly	1.73	1.51	1.47	1.24	1.56								1.2	
Pro	4.21		2.8	2.35	3.07								2.3	
Ser	2.27	1.43	1.5	1.42	1.54								1.2	
Tyr	2.2	1.33	1.52	1.19	1.21								1.2	
Lys:CP ratio	2.2	3.63												
Energy, kcal/kg														
GE		5397.58	5153.17	5720.19	5660.54									5161.66

2. PORCINE *IN VITRO* DEGRADATION CHARACTERISTICS OF HEAT PRETREATED CORN WHOLE STILLAGE

ABSTRACT. Pretreatment of whole stillage (WS; slurry material that is dried into DDGS) with heat can improve digestibility of the resulting DDGS by pigs. A study was conducted to identify optimal conditions (time and temperature) for heat pretreatment of corn WS. Six samples of WS from different sources were divided into 13 sub-samples to give a total of 78 sub-samples. Thirteen treatments were applied to 13 sub-samples from each source (1 sub-sample/treatment). The treatments were untreated WS, and WS that was pre-treated (70 psi) for 10, 20, or 30 min and at 100, 120, 140, or 160 °C in a 3 × 4 factorial arrangement. Sub-samples were subjected to *in vitro* digestion with porcine pepsin and pancreatin, followed by *in vitro* fermentation for 72 h. Accumulated gas production was recorded and modeled to estimate kinetics of gas production. Furans and AA contents were measured in the feedstuffs. Starch and dietary fiber components contents were measured in the feedstuffs and undigested residues. Pretreatment time and temperature did not interact on *in vitro* digestibility of DM (IVD-DM), and total gas. The IVD-DM for untreated WS was 73.4%. An increase in pretreatment temperature from 100 to 160 °C resulted in linear and quadratic increase in IVD-DM by 11%. Response surface analysis indicated that maximum IVD-DM resulted from relatively long pretreatment times (20 to 30 min) and highest pretreatment temperature. A rise in pretreatment temperature from 100 to 160 °C resulted in linear increase in total gas production by 13%; maximum total gas production resulted from relatively short pretreatment times (10 to 20 min) and highest pretreatment temperature. Furans levels increased primarily at 160° over 20 min. Interactions were observed in dietary fiber composition. The maximum for available Lys was found at 101 °C at 20 min. In conclusion, the optimal conditions for pretreatment of

WS for production of DDGS that is highly digestible and fermentable by pigs were temperatures of between 140 and 160°C, and duration of approximately 20 min; nevertheless AA composition of the WS might be negatively affected at these pretreatment conditions.

2.1. INTRODUCTION

Corn DDGS is the most important co-product of the global biofuel industry and it is nowadays widely used in animal feeds (Shurson, 2017). As a result of the alcoholic fermentation of corn where most of the starch is converted in ethanol, DDGS has a low starch content and approximately 3 times greater oil, NSP and protein content than corn (Liu, 2011). Therefore, corn DDGS is a source of energy and amino acids for monogastric animals (ŚWiĄTkiewicz and Koreleski, 2008; Stein and Shurson, 2009). Notwithstanding, DDGS utilization in formulation of monogastric diets is limited to some extend by its high content of NSP (~30%), which are poorly digestible and can reduce dietary nutrient utilization by: (1) reducing digestibility of nutrients by encapsulation, (2) increasing endogenous nutrient losses, and (3) increasing passage rate of digesta in gastrointestinal tract (Noblet and Le Goff, 2001; Bedford, 2018). Furthermore, DDGS is subjected to heat during the drying step of its production, which can lead to Millard reactions and therefore affect the availability of amino-acids, particularly lysine (Almeida et al., 2013; Teodorowicz et al., 2018). It is therefore of great interest for the animal feed industry to develop and evaluate technologies that alleviate the negative effects of NSP in DDGS, resulting in enhanced utilization of DDGS by pigs (de Vries et al., 2012). Several processing methods have been suggested to enhance nutritive value of DDGS. Typical post-manufacture processing techniques such as grinding, pelleting, and extruding have

been suggested to enhance nutritive value of DDGS. However, these techniques have not shown considerable improvements particularly with regard to reducing the anti-nutritional effects of NSP (de Vries et al., 2012; Rojas and Stein, 2017). In addition to the fore-mentioned post-manufacture processing techniques, pretreatment methods that are used in process of producing ethanol from lignocellulose materials have been suggested to enhance nutritive value of DDGS (de Vries et al., 2013; Zangaro et al., 2018). Pretreatment of lignocellulosic material results in disruption recalcitrant fiber structures, thereby making the biomass available for enzymatic digestion and hence ethanol yield (Amin et al., 2017). Zangaro et al. (2018) determined the effects of heat pretreatment of WS at 160 °C and 70 psi for 20 min on digestibility and fermentability of the same feedstuff using a porcine *in vitro* model and observed a 16% improvement in the digestibility due to the pretreatment. Pretreatment technologies can be incorporated into the ethanol production process in bioethanol plants for enhancing value of coproducts (Chatzifragkou et al., 2015). The nutritive value and cost of pretreated WS for pigs is dependent on conditions for the pretreatment of the WS. However, information is lacking on optimal conditions for pretreatment of WS for pigs. Objective of this study was identified optimal the conditions (time and temperature) of heat pretreatment of WS for pigs using a porcine *in vitro* digestion and fermentation model.

2.2. MATERIALS AND METHODS

2.2.1. Sample Source and Treatment Arrangement

A sample of WS from different six different ethanol plants (1 sample per plant) in South Dakota and Minnesota were obtained and divided into 13 sub-samples to give a total of 78 individual sub-samples. Thirteen treatments were applied to 13 sub-samples from

each source (1 sub-sample/treatment). The treatments were untreated WS, and WS that was pre-treated (70 psi) for 10, 20, or 30 minutes and at 100, 120, 140, or 160 °C in a 3 × 4 factorial arrangement.

2.2.2. Heat and Pressure Treatment

The samples were heat-pretreated at the National Center for Agricultural Utilization Research (NCAUR) in Peoria, IL. Briefly; 500 ml of WS was added to a 500 ml working volume stainless steel reactor (2" diameter sanitary tubing with fluoroelastomer rubber gaskets, end caps, and bolted high pressure sanitary clamps) then placed in a Techne Industrial Fluidized Sand Bath (model IFB-101, Techne Incorporated, Burlington, NJ). Reactor temperature was monitored using an internal thermocouple probe and brought to 160 °C then held at the target temperature 20 minutes. Reactor was immediately cooled by transferring it to a vessel containing cold water. The pretreated WS was then transferred to individual Nalgene bottles (1 L), frozen and shipped to the Department of Animal Science at the South Dakota State University.

2.2.3. Porcine in vitro Digestion

The 78 subsamples (13 samples per ethanol plant) of WS were freeze dried and ground to pass through a 0.75 mm screen using an ultra-centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany). Subsequently, the samples were subjected to *in vitro* digestion with porcine pepsin and pancreatin as described by Woyengo et al. (2016). Briefly, 4 grams of samples were weighed into 500 mL Erlenmeyer flask. A phosphate buffer solution (200 mL, 0.1 M, pH 6.0), HCl solution (80 mL, 0.2 M) and fresh pepsin (4 mL, 20 g/L porcine pepsin, P-0609; Sigma-Aldrich Corp., St. Louis, MO, USA) were then added into the flasks with the samples. Additionally, 2 mL of chloramphenicol (C-0378; Sigma-Aldrich Corp., St. Louis, MO, USA) solution (0.5 g/100 mL) was added in the flasks to prevent bacterial

growth during the enzymatic hydrolysis. The samples were then placed into a water bath at 39 °C for 2 h under a gentle agitation (50 revolutions/minute). After pepsin hydrolysis, phosphate buffer solution (80 mL, 0.2 M, pH 6.8), NaOH (20 mL, 0.6 M), and fresh pancreatin solution (8 mL, 100 g/L pancreatin; P-1750 Sigma-Aldrich Corp., St. Louis, MO, USA) were added into the flasks, and digestion was continued for 4 h in water bath at the same conditions under which the samples were digested with pepsin. The residues of the samples after the digestion were collected by filtration on a nylon cloth (50 μm), and then washed with ethanol (2 × 25 mL 95% ethanol) and acetone (2 × 25 mL 99.5% acetone). The washed residues were dried for 12 h at 60 °C and weighed for determination of *in vitro* digestibility of DM (**IVD-DM**).

Three sets of *in vitro* digestion were performed for each treatment in order to generate enough residue to perform an *in vitro* fermentation experiment. The experimental scheme was as follows: (13 treatments × 6 batches × 3 cycles), each batch correspond to one randomly selected set of samples from the same ethanol plant source. The experimental design was a randomized complete block design with batch as a blocking factor. The undigested residue from the three cycles was pooled for each treatment for further *in vitro* fermentation.

2.2.4. Porcine in vitro Microbial Fermentation

The undigested residues were subjected to porcine *in vitro* fermentation using fresh pig feces as inoculum, and a cumulative-gas production technique as describe by Zangaro et al. (2018). Briefly, samples were placed in anaerobically sealed bottles and incubated a water bath at 39 °C with a slight agitation of 50 rpm. The gas pressure generated during fermentation in each bottle was measured at 0, 2, 5, 8, 12, 18, 24, 36, 48, and 72 h using a

pressure transducer (SIN-54978; GP:50, Grand Island, NY) that was fitted with a digital data tracker (Blue Ribbon Corp., Grand Island, NY), followed by a complete ventilation using an 18 g × 1" hypodermic sterile needle after each recording time. After 72 h of incubation, fermentation was stopped by placing the bottles in ice. The contents of the bottles were transfer to 50mL conical centrifuge tubes and stored in a −20 °C freezer. The experimental scheme for *in vitro* fermentation was as follows: three batches of in-vitro fermentation were conducted, where each batch contained the complete set of duplicated samples from two randomly selected ethanol plants (2×13×2), accompanied by 4 bottles containing only the reagents to serve as sample blanks and three bottles containing inulin as a control for the fermentation, to give a total of 59 bottles per fermentation which fulfilled the water bath capacity.

Animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (Approval No. 15-069E).

2.2.5. Sample Analyses

The ground samples and residues from *in vitro* digestion were analyzed for DM (method 930.15) and crude protein (CP, method 984.13) according to AOAC (2012). Soluble dietary fiber (**SDF**), and insoluble dietary fiber (**IDF**) contents of untreated and pretreated WS were measured by using the Megazyme Total Dietary Fiber kit (Megazyme International Ireland Ltd, Wicklow, Ireland) according to AOAC-991.43 and AACC-32-07.01 methods (AOAC, 2012; McCleary et al., 2012). Total furans, furfural and HMF contents in untreated and pretreated WS samples were measured using high performance liquid chromatography (**HPLC**); the analyses were conducted by Celignis Ltd, University

of Limerick, Ireland. Untreated and pretreated WS samples were analyzed for amino acids contents (method 975.44; 982.30 AOAC, 2006) and for available lysine (method 45.3.05; AOAC, 2006) at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO).

2.2.6. Calculations

Total dietary fiber content (**TDF**, g/100g) was calculated as the sum of IDF and SDF content in the analyte. Lysine as proportion of CP (g/100g) in feedstuffs was calculated by dividing the lysine content by the CP content followed by multiplying the resulting quotient by 1,000. The *in vitro* disappearance (**IVD**) of DM and nutrients after pepsin and pancreatin digestion was calculated as follows:

$$IVD\ (g/100g) = \frac{\text{weight of intact sample} - \text{weight of residue}}{\text{weight intact sample}} \times 100$$

Gas pressure measurements were converted into gas volume (G, per gram DM) using the ideal gas law, assuming an atmospheric pressure of 101,325 Pa and a temperature of 312.15 K. Gas accumulation curves recorded during the 72 h of fermentation were modelled according to France et al. (1993):

$$G\ (mL\ g^{-1}\ DM)\ = 0, if\ 0 < t < L$$

$$G\ (mL\ g^{-1}\ DM)\ = G_f\left(1 - \exp\{-(b(t-L) + c\left(\sqrt{t} - \sqrt{L}\right)\right\}, if\ t \geq L$$

where, G denotes the gas accumulation to time, G_f (mL/g DM) the maximum gas volume for t = ∞ and L (hours, h) the lag time before the fermentation starts. The constants b (h−1) and c (h-1/2) determine the fractional rate of degradation of the substrate μ (h−1), which is postulated to vary with time as follows:

$$\mu = b + \frac{c}{2\sqrt{t}}, t \geq L$$

Kinetics parameters (Gf, L, μt=T/2 and T/2) were compared in the statistical analysis. The T/2 is the time to half-asymptote when G=Gf/2.

2.2.7. Statistical Analyses

The effects of heat pretreatment on *in vitro* digestion of DM, amino acid profile, available lysine, lysine to CP ratio, total furans, furfural and HMF and parameter estimates of the gas production modeling during *in vitro* fermentation were subjected to analysis of variance using the MIXED procedure of SAS (SAS Studio, SAS Institute Inc., Cary, NC). The experimental design was a randomized complete block design with a 3 × 4 factorial arrangement with time and temperature as factors. Batch was considered as the block and flask (for *in vitro* digestion) or glass bottle (for *in vitro* fermentation) as the experimental unit. The model included time and temperature as the fixed effects factors and batch as a random effect factor. The fixed effects were tested by using Type 3 estimable functions for temperature, time and their interactions. Model residuals were tested for homogeneity and normality. Least squared means were determined for interaction terms and main effects when appropriate. Treatment means were separated by the probability of difference when interaction between time and temperature resulted significant. When interaction term resulted insignificant, a multiple comparison with Dunnett-Hsu adjustment (compare each of a number of treatments with a single control; untreated sample as control) was perform by each level of the Least squared mean effect. Total furans, furfural and HMF data were normalized (before statistical analysis) using the log transformation because several samples resulted in undetectable levels of furans. The resulting least squared means were back transformed for presentation of the results. Significance level was set at $P \leq 0.05$ for all statistical tests.

PROC RSREG of SAS was used to fit a response surface model to the time and temperature corrected for the source of WS in order to estimate the optimum value of IVD-DM and total gas production (**TGP**) and the correspondent time and temperature conditions. The sample ethanol plant source was considered as a covariate of the regression model to fit a response surface to the dependent variables corrected for the covariates. Furthermore, a ridge analysis was used to determine the region in which the optimum lies when the canonical analysis of the response surface resulted in a saddle point (non-single optimum value).

2.3. RESULTS

Table 2.9 presents the effects of heat pretreatment of WS on starch, TDF, SDF and IDF contents of WS. Untreated WS had lower ($P < 0.05$) starch content and greater ($P < 0.05$) TDF, IDF and SDF contents than pretreated WS. Pretreatment time and temperature interacted ($P < 0.05$) on starch content in WS such that an increase in pretreatment temperature resulted in an increase ($P < 0.05$) in starch content in WS, and that an increase in duration of pretreatment reduced ($P < 0.05$) starch content in WS when the latter was pretreated at 100 or 120 °C, but increased ($P < 0.05$) the starch content in the WS when the latter was pretreated at 160 °C. Pretreatment time and temperature interacted on TDF content in WS such that an increase in pretreatment temperature resulted in a decrease ($P < 0.05$) in TDF content in WS, and that an increase in duration of pretreatment did not affect TDF of WS when the latter was pretreated at 100, 120 or 140 °C, but reduced ($P < 0.05$) the TDF content in the WS when the latter was pretreated at 160 °C. Pretreatment time and temperature interacted on IDF content in WS such that an increase in pretreatment temperature resulted in a decrease ($P < 0.05$) in IDF content in WS, but an increase in

duration of pretreatment did not affect IDF of WS at any level of temperature. Pretreatment time and temperature interacted on SDF content in WS such that an increase in pretreatment temperature resulted in a decrease ($P < 0.05$) in SDF content in WS, and that an increase in duration of pretreatment did not affect SDF of WS when the latter was pretreated at 100 or 120 °C, but reduced ($P < 0.05$) the SDF content in the WS when the latter was pretreated at 140 or 160 °C.

Table 2.10 present the effects of heat pretreatment on the amino-acid profile, CP, available Lys and the Lys as proportion of CP values of WS. Untreated WS had a greater ($P < 0.01$) content of Arg, His, Lys, Met, Phe, Thr and Trp than WS that had been pretreated at 140 or 160 °C for 20 or 30 min. There was an interaction ($P < 0.05$) between time and temperature on the content of most indispensable amino acids in WS such that the higher pretreatment temperatures (140 and 160°C) and longer times (20 and 30 min) resulted in lower levels of the tested amino acid, but the magnitude of the reduction in amino acid content in WS was greater at 160 °C than at 140 °C. With regard to dispensable amino acids, the content of Asp, Cys and Pro in WS was negatively affected by heat pretreatment and the interactions between pretreatment temperature and time on the content of these amino acids in WS were like that fore-mentioned for indispensable amino acids. Nevertheless, the level of CP in WS was not affect by the pretreatment conditions. The level of available Lys and Lys to CP in WS were reduced ($P > 0.05$) by the heat pretreatment. Pretreatment time and temperature interacted on available Lys and Lys to CP in WS such that pretreatment at 100 °C did not affect these response criteria, whereas pretreatment at 120, 140 or 160 °C reduced the available Lys and Lys to CP values in WS; and that an increase in pretreatment time from 10 to 30 min reduced the available Lys and

Lys to CP values in WS when the WS was pretreated at 140 or 160 °C, but not when the WS was pretreated at 100 or 120 °C.

Table 2.11 shows the effects of heat pretreatment of WS on total furans, furfural and HMF content in WS. Pretreatment time and temperature interacted on total furans and HMF content in WS such that an increase in pretreatment temperature resulted in an increased ($P < 0.05$) in total furans content in WS, and that an increase in duration of pretreatment did not affect total furans content of WS when the latter was pretreated at 100, 120 or 140 °C, but increased ($P < 0.05$) the total furans content in the WS when the latter was pretreated at 160 °C. Pretreatment time and temperature did not interact on furfural content in WS. An increase in pretreatment temperature resulted in an increase ($P > 0.05$) in furfural content in the WS. Also, an increase in pretreatment time tended to result in an increase ($P = 0.08$) in furfural content in WS.

Table 2.12 presents the effects of heat pretreatment of WS on IVD of DM, starch, TDF, IDF and SDF values of WS. Time and temperature did not interact ($P >0.05$) on IVD of DM. However, the IVD of DM was increased ($P <0.05$) with an increase in pretreatment temperature. Pretreatment time and temperature interacted ($P < 0.05$) on IVD of starch value of WS such that an increase in pretreatment temperature resulted in an increase ($P < 0.05$) in IVD of starch of WS, and that an increase in duration of pretreatment reduced ($P < 0.05$) IVD of starch of WS when the latter was pretreated at 100 or 120 °C, but increased ($P < 0.05$) the IVD of starch of WS when the latter was pretreated at 160 °C. The IVD of TDF, IDF and SDF for the WS was not affected by pretreatment temperature or duration.

The treatment effects on *in vitro* fermentation kinetics parameters are presented in Table 2.13. The lag time was not affected by the pretreatment time and temperature.

Pretreatment time and temperature interacted on half-time to asymptote such that an increase in pretreatment temperature from 120 to 160 °C resulted in a decrease ($P < 0.05$) in half time parameter, and that an increase in duration of pretreatment time did not affect the half time to asymptote at pretreatment temperature of 100 or 120 °C, but decreased ($P < 0.05$) the half time to asymptote at pretreatment temperature of 140 or 160 °C. The degradation rate followed a trend similar to that of half-time parameter. Time and temperature conditions did not interact ($P > 0.05$) on TGP. Also, pretreatment time did not affect TGP. However, an increase in pretreatment temperature resulted in an increase ($P < 0.05$) in TGP.

The dependent variables IVD-DM, TGP and available Lys were selected to fit a response surface model due to their biological importance; the pretreatment time and temperature were independent variables. The fitted response surfaces are presented in Figure 2.5. The surface models for the three responses were significant ($P < 0.001$) with an R^2 values of 0.79, 0.41 and 0.98 for IVD-DM, TGP and available Lys, respectively. The canonical analysis of the surfaces indicated that stationary points in the surfaces were saddle points, which indicate that the estimated surfaces do not have a unique optimum. However, the ridge analysis (Figure 2.6) indicated that maximum yields of IVD-DM and TGP resulted from relatively high pretreatment temperatures (~159°C) and medium pretreatment duration (~20 min), whereas the maximum available Lys content was achieved at low pretreatment temperatures (~100°C) and medium pretreatment duration (~20 min).

2.4. DISCUSSION

In this study, samples of corn WS were obtained from 6 different ethanol plants in order to take care of variability in composition of WS (and hence DDGS) that is due to differences

in ethanol and DDGS production conditions among ethanol plants (Spiehs et al., 2002). The starch content of the untreated WS samples were similar to previously reported values for WS (Han and Liu, 2010; Liu, 2011). Starch content in WS was increased with an increase in pretreatment temperature. As previously mentioned, WS is the slurry material that remain after alcoholic fermentation of corn grain that has been digested with starch-hydrolyzing enzymes. Starch that escape digestion by starch-hydrolyzing enzymes in the small intestine of animals is known as resistant starch (Englyst et al., 1992). The starch present in WS is starch that escape enzymatic digestion during ethanol production from corn grain, and hence it could be resistant starch. A variety of types of resistant starch might be found on DDGS samples and their occurrence is related to different processes such as drying, gelatinization or encapsulation (Li et al., 2014). Some studies have reported increased levels of starch in corn, pea and lentils as a result of moisture-heat treatment of resistant starch (Sang and Seib, 2006; Chung et al., 2009; Ozturk et al., 2009). This increase in starch content in feedstuffs due to moisture-heat treatment is likely due to the denaturalization of proteins that might impede the exposure of starch to digestive enzymes, and it may explain the observed increase in starch content of the pretreated WS in the current study due to the increase in pretreatment temperatures. The WS has residual of thermostable amylase activity (Singh et al., 2006). Thus, the observed decrease in WS starch content due to an increase in pretreatment period at lower pretreatment temperatures (100 and 120 ° C) could be attributable to the fact that starch was continued to be hydrolyzed by the residual thermostable amylases under these conditions.

The dietary fiber composition (TDF, IDF and SDF) of WS has not been previously reported. Nonetheless the ratio of TDF to IDF and SDF values of the untreated WS were similar to

those reported for corn (Jaworski and Stein, 2017; Navarro et al., 2018). High proportion of TDF in corn is IDF. For instance, the IDF content as proportion of TDF in corn was 88% (Jaworski and Stein, 2017). The average IDF content as proportion of TDF in untreated WS used in the current study was 88.4%, which is explained by the high IDF content (as proportion of TDF) in the parent corn grain. Heat pretreatment of WS resulted in reduced of dietary fiber components. Past studies on hydrothermal pretreatment (pretreatment with heat and pressure in the presence of water) have shown reduction in molecular weight of pretreated fibers indicating substantial thermochemical depolymerization (Merali et al., 2013). During hydrothermal pretreatments, water is auto ionized into acidic hydronium ions (H_3O^+) that act as catalysts in the hydrolysis of the glycosidic bonds of NSP. Hydronium ions are generated during the cleavage of bonds between the xylose units (of backbone chains of arabinoxylans) and uronic acids (of side chains of arabinoxylans), which further contribute to hydrolysis of hemicellulose into oligosaccharides or monomeric sugars (Mosier et al., 2005b; Chatzifragkou et al., 2015). This mechanism of action therefore can explain the reduced content of dietary fiber components in pretreated WS. Zangaro et al. (2018), similarly reported reduced fiber (NSP) content in WS due to heat pretreatment. In the present study the magnitude of change in IDF content of WS as consequence of heat pretreatment was different from the magnitude of change in SDF of WS due to heat pretreatment (14 vs. 30%). Chacng and Morris (1990) reported reduced SDF content, but not IDF content of corn due to autoclaving of the corn. Therefore, SDF fraction is more susceptible to heat treatments than IDF. On the other hand, as previously mentioned, hydrothermal treatment can disrupt NSP structures, especially those of hemicellulose such as arabinoxylans found in corn, leading to solubilization of IDF. For

instance, Wang et al. (2018) observed reduced IDF content and increased SDF content without significant change in TDF content in defatted corn hulls due to hydrothermal treatment of the defatted corn hulls, implying that the hydrothermal treatment resulted in conversion of IDF into SDF. Therefore, it had been assumed that heat pretreatment of WS in the current study would result in reduced IDF content and increased SDF content of the WS. However, both IDF and SDF fractions of the WS were reduced by the pretreatment, leading to reduced TDF content of the WS. The SDF of corn fiber can be classified in two types based on molecular weight; low-molecular-weight soluble dietary fiber and high-molecular-weight soluble dietary fiber (McCleary, 2014; Wang et al., 2018). Increased temperature (in hydrothermal treatment) can result in secondary decomposition of SDF into lower molecular weight fractions (Wang et al., 2018). Therefore, the observed reduction in SDF content of WS might partly be explained by conversion of the SDF into low molecular weight SDF. The DF determination method used in the current study might underestimate low molecular weight soluble dietary fiber (Ramulu and Rao, 1997). Finally, although hydrothermal treatments primarily lead to depolymerization of NSP to oligosaccharides (Chatzifragkou et al., 2015), formation of monosaccharides might occur as well and exacerbate as temperature of pretreatment increases (Samala et al., 2012).

The amino acid profile of the untreated WS samples is similar that which was reported by Han and Liu (2010) for corn WS. This is the first study to report amino acid composition of corn WS that has been subjected to heat pretreatment. Heat treatment of feedstuffs can lead to Maillard reactions, resulting reduction in amino acid content of the feedstuffs (Pahm et al., 2008). Kim et al. (2008) reported reduced content of Cys, Arg and Lys (but not of other amino acids) in corn DDGS due to liquid hot water pretreatment or ammonia fiber

expansion. In the current study, heat pretreatment reduced Arg, His, Lys, Trp and Cys content in WS. The most affected amino acid was Lys; its content in the WS was reduced by 36% due to the pretreatment. Zangaro et al. (2018), did not observe a change in Lys content of WS due to pretreatment of the WS with heat at 70 psi and 160 °C for 20 min. However, in the current study, Lys content in WS was reduced from 1.03 to 0.72% due to pretreatment of WS with heat at the same conditions at which WS was pretreated with heat in the study of Zangaro et al. (2018). Lysine is the first limiting AA in practical swine diets (Liao et al., 2015). Therefore, special attention is paid to content of this AA in swine feedstuffs. Furthermore, Lys is more susceptible to heat damage (than most of the other amino acids) due to Maillard reactions (Pahm et al., 2008). Therefore, susceptibility of certain AA like Lys to heat treatment might be a potential limitation of heat pretreatment of WS.

Apart from the reduced Lys content, heat treatment can result in reaction of Lys with other compounds, leading to reduced bioavailability of the Lys. Conventional wet chemistry methods of analysis of amino acids involve hydrolysis of samples with a strong acid, which liberate Lys that is otherwise not bioavailable, leading to a significant overestimation of bioavailable Lys in heat treated feedstuffs (Fontaine et al., 2007). Thus, available Lys is a better estimator of Lys that is bioavailable in feedstuffs (Moughan and Rutherfurd, 2008). The available Lys content of corn WS has not been reported. In the current study, available Lys content in WS decreased by up to 55% as consequence of heat pretreatment. Similarly, the available Lys content in corn DDGS was reduced due to autoclaving of the DDGS (Almeida et al., 2013). Notwithstanding, the dramatic reduction in the available Lys content observed in this study, it should be noted that traditional DDGS

is exposed to high temperatures during the drying process and is therefore not uncommon to find low level of available Lys in corn DDGS.

Furfurals and HMF are produced from monosaccharides during pretreatment of fibrous feedstuffs with water, alkalis or acids at high pressure and temperature (Steinbach et al., 2017). The production of furfurals and HMF is often evaluated as part of optimization of pretreatment technologies for second generation ethanol production (Mosier et al., 2005a; Yan et al., 2016; Iram et al., 2019). This is because furfurals and HMF can inhibit the ability of bacteria or yeast to ferment sugars to ethanol (Mosier et al., 2005c). Also, furfurals and HMF can reduce cellulolytic and hemicellulolytic activity by enzymes (Panagiotou and Olsson, 2007; Jing et al., 2009). Liquid hot water pretreatment of feedstuffs compared with alkali or acid pretreatment results in lower polysaccharide hydrolysis to monosaccharides, leading to lower production of these toxic compounds (Mosier et al., 2005a; Mosier et al., 2005c; Kim et al., 2008; Yan et al., 2016). The conditions at which WS was pretreated in the current study are similar to the conditions at which feedstuffs are subjected to during LHW pretreatment because LHW pretreatment involves addition of water to the feedstuffs followed by its pretreatment at high temperature and pressure, whereas in the current study the WS that was already high in moisture content (~90%) was pretreated with heat at high temperature and pressure. The generation of furans in the WS was increased as a result of heat pretreatment. Nevertheless, data had to be transformed in order to detect the effects of pretreatment because the level of furans was not detectable levels in some samples, leading to a skewed distribution of furan values. Furfural and HMF were observed primarily in WS that was pretreated at the higher temperatures (140 or 160°C). However, the furfural and HMF values for the WS that was

pretreated at the high temperature were comparable to those that were observed by (Almeida et al., 2013) in conventional DDGS. The production of furfural and HMF represent a challenge for cellulosic ethanol production (Modig, 2002) and from the swine nutrition point of view these compounds can be used as indicators of Maillard reactions and hence quality of protein in feedstuffs. Also, they can inhibit activity of digestive enzymes and reduce microbial fermentation in ethanol production (Almeida et al., 2009). Additionally, these compounds had hepatotoxic and hepatocarcinogen effects in rats that had consumed them at ≥2 mg/kg BW (Moro et al., 2012), implying that they can be toxic to animals and humans. However, the effects of dietary furfural and HMF on gastrointestinal digestive enzyme activities and organic matter fermentation and hence nutrient digestibility in pigs or poultry has not been reported. Also, the toxicity and safe dietary level of these compounds in pigs and poultry have not been reported.

In the current study, the IVD of DM was improved as a result of heat pretreatment, which might be explained by the increased IVD of starch and partial solubilization of fiber into simple sugars as evidenced by the reduction in the TDF, IDF and SDF levels in WS due to the pretreatment. Simple sugars are highly digestible. The increase in IVD of starch due to the heat pretreatment can be attributed to the fact that the pretreatment can disrupt the matrix structure of fiber, leading to increased availability of fiber-encapsulated nutrients including starch for digestion. Results from this study are similar to those from a previous study (Zangaro et al., 2018) in which pretreatment of WS with heat increased IVD of DM. An increase in IVD of starch was observed when WS was pretreated at 140 or 160 °C; at these pretreatment temperatures the IVD of starch was also increased with increase in pretreatment duration. An increase in the susceptibility of starch to enzymatic hydrolysis

as a result of heat-moisture treatment has been observed in waxy, normal, and high-amylose cornstarch (Franco et al., 1995) and granular cornstarch Kong et al. (2018). Hydrothermal treatment can alter the physicochemical properties of starch without destroying its granular structure, and promote crystalline disruption and the dissociation of double helical structures in the amorphous region; these can facilitate the attack of α-amylase within the starch granules (Zavareze and Dias, 2011). The observed increase in digestibility of starch due to pretreatment of WS at higher temperature and for longer period might therefore be attributed to the increased disruption of starch structure and hence its increased susceptibility to enzymatic hydrolysis. In the current study, an increase in pretreatment period led to a decreased IVD of starch when the WS was pretreated at 100 or 120 °C. Chung et al. (2009), reported a decrease in rapid digestible starch content and an increase in slowly digestible starch and resistant starch (**RS**) contents of cornstarch when the cornstarch was heat-moisture treated at 120 °C. Thus, the decrease in digestibility of starch in WS that was pretreated at 100 or 120 °C due to an increase in the pretreatment period may be due to creation of slowly digestible starch and RS in WS due to pretreatment at these conditions. The IVD of TDF in this study are similar to those of DDGS reported by Huang et al. (2017). Since pigs do not produce enzymes that can degrade fiber, no further disappearance of fiber components is expected to occur during the simulated gastric and small intestinal phases of digestion, and this could explain why the IVD of TDF was unaffected by the pretreatment.

An increase in pretreatment temperature and not pretreatment time resulted in increased TGP, which is an indicator of the extent of fermentation of the undigested WS residue in the large intestine of pigs. Also, heat pretreatment reduced half time and

increased rate of degradation of undigested residue, implying that heat pretreatment of WS can increase the rate of hindgut fermentation of small intestinal undigested residue of WS. The increased rate and extent of fermentation of WS due to the pretreatment can be attributed to disruption of carbohydrate-lignin matrix structure and depolymerization of NSP into readily fermentable short fragments. Zangaro et al. (2018), did not observed significant changes in fermentation kinetic parameters of WS due to pretreatment of the WS at 160 °C for 20 min. The reason for the difference between the current study and that of Zangaro et al. (2018) with regard to fermentation kinetics is not clear. Hydrothermal pretreatment has been used in the past to improve biogas production from lignocellulosic materials (Chandra et al., 2012a; Chandra et al., 2012b; Papa et al., 2015). Therefore, pretreatment of WS is expected to have an accelerating effect on fermentation because of the generation of readily fermentable fractions in the biomass.

The optimization analyses (response surfaces and ridge analysis) should be performed using biologically relevant characteristics of the test feedstuff. For heat treated fibrous feedstuff like WS, the most biologically relevant characteristics are small intestinal and large intestinal digestibility, and protein quality. Thus, IVD of DM, TGP and available Lys content were the variables used to perform optimization analyses. This approach illustrated the need for compromises in any attempt to simultaneously optimize the IVD of DM, TGP and available Lys. Pretreatment at higher temperatures (140 or 160 °C) for medium duration (~20 min) resulted optimum digestibility and fermentation. However, available Lys content in the WS was reduced at these pretreatment conditions (140 or 160 °C for ~20 min); the maximum available Lys content in the WS was observed when the WS was pretreated at ~100 °C and 20 min. In a similar study, the economic analysis performed by

Perkis et al. (2008) of modified DDGS (pretreated with AFEX method) indicated no improvements in profitability for the ethanol plant; although the modified DDGS had a higher protein levels, the DDGS prices were more sensitive to the amino acid profile than total protein levels, and the modified DDGS had lower level of Lys, which is the most limiting AA in practical swine diets. Therefore, economic and animal performance evaluations of heat pretreated WS are warranted.

2.5. CONCLUSION

Heat pretreatment of WS might be an effective way to improve the nutritive value of the resulting DDGS for pigs, because the disruption of DF components as a result of the hydrothermal treatment resulted in improved digestibility and fermentability of the pretreated feedstuff. The optimal conditions for pretreatment of WS for production of DDGS that is highly digestible and fermentable by pigs were temperatures of between 140 and 160°C, and duration of approximately 20 min. However, protein quality of DDGS is lowered when the WS is pretreated at the fore-mentioned conditions. Thus, further research is warranted to investigate trade-offs between energy value and protein quality of pretreated WS for pigs and the effects of pretreating WS with heat at commercial scale on nutritive value for pigs and poultry.

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Table 2.9. Effects of changes in time and temperature (TEMP) conditions of heat pretreatment on starch, total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) content of whole stillage

Factor		***Analyte, g/100 g***			
TEMP, °C	**TIME, min**	**Starch**	**TDF**	**IDF**	**SDF**
0	0	0.93^{fgh}	28.18^{a}	24.91^{a}	3.27^{a}
100	10	1.03^{g}	26.88^{bc}	23.99^{ab}	2.89^{b}
	20	0.79^{h}	27.25^{ab}	24.33^{a}	2.92^{b}
	30	0.56^{i}	27.62^{ab}	24.66^{a}	2.96^{b}
120	10	1.24^{e}	26.23^{d}	23.47^{c}	2.76^{c}
	20	1.14^{f}	26.28^{d}	23.54^{c}	2.75^{c}
	30	1.04^{g}	26.33^{cde}	23.60^{bcd}	2.73^{cd}
140	10	1.45^{d}	25.58^{ef}	22.95^{de}	2.63^{d}
	20	1.49^{d}	25.32^{fg}	22.74^{ef}	2.57^{e}
	30	1.53^{cd}	25.05^{fg}	22.54^{ef}	2.51^{f}
160	10	1.66^{c}	24.93^{g}	22.43^{fg}	2.51^{ef}
	20	1.84^{b}	24.35^{h}	21.95^{g}	2.39^{g}
	30	2.02^{a}	23.76^{i}	21.48^{g}	2.28^{h}
SEM		0.148	0.148	0.312	0.315
P - value					
TEMP		<.0001	<.0001	<.0001	<.0001
TIME		0.011	0.794	0.723	0.522
TEMP×TIME		<.0001	<.0001	0.001	<.0001

Table 2.10. Effects of changes of time and temperature (TEMP) conditions of heat pretreatment on amino acid (AA) profile, crude protein (CP) content, available Lys content, and Lys to CP ratio of whole stillage

Factor		***Analyte, g/100 g***									
		Indispensable AA									
TEMP, °C	**TIME, min**	**Arg**	**His**	**Ile**	**Leu**	**Lys**	**Met**	**Phe**	**Thr**	**Trp**	**Val**
0	0	1.24^{abcd}	0.76^{abcd}	1.07	2.95	0.98^{abc}	0.51	1.60^{a}	1.05^{a-h}	0.20^{a-k}	1.37
100	10	1.29^{a}	0.76^{c}	1.08	3.02	0.97^{c}	0.51	1.60^{a}	1.07^{ac}	0.22^{ae}	1.39
	20	1.31^{a}	0.77^{b}	1.07	3.02	1.01^{b}	0.51	1.60^{abcde}	1.07^{a}	0.23^{abcd}	1.39
	30	1.33^{a}	0.78^{a}	1.07	3.02	1.04^{a}	0.51	1.60^{ab}	1.07^{ab}	0.23^{abcd}	1.39
120	10	1.25^{b}	0.75^{d}	1.08	3.03	0.92^{d}	0.5	1.60^{abd}	1.06^{abcd}	0.21^{bf}	1.39
	20	1.23^{bc}	0.74^{d}	1.07	3.02	0.91^{d}	0.5	1.60^{abc}	1.06^{bc}	0.21^{fgh}	1.39
	30	1.22^{bcd}	0.74^{de}	1.06	3.01	0.90^{de}	0.5	1.59^{ab}	1.06^{cdef}	0.21^{efhi}	1.38
140	10	1.22^{c}	0.73^{e}	1.07	3.04	0.87^{e}	0.5	1.59^{b}	1.06^{abcd}	0.21^{cg}	1.39
	20	1.16^{e}	0.72^{f}	1.06	3.02	0.82^{f}	0.5	1.59^{a-e}	1.05^{e}	0.20^{i}	1.38
	30	1.10^{f}	0.70^{g}	1.06	3	0.77^{g}	0.5	1.58^{bc}	1.04^{gh}	0.19^{jk}	1.38
160	10	1.18^{de}	0.72^{ge}	1.07	3.05	0.82^{f}	0.5	1.57^{cef}	1.05^{a-g}	0.20^{dhij}	1.39
	20	1.08^{f}	0.69^{g}	1.06	3.02	0.72^{h}	0.49	1.56^{def}	1.04^{fh}	0.18^{k}	1.38
	30	0.99^{g}	0.66^{i}	1.05	2.99	0.63^{i}	0.49	1.55^{f}	1.02^{i}	0.16^{l}	1.38
SEM		0.05	0.04	0.04	0.17	0.07	0.03	0.03	0.03	0.02	0.05
P - value											
TEMP		<.0001	<.0001	0.4363	0.9848	<.0001	0.181	0.3554	0.0079	0.0024	0.8327
TIME		0.0072	0.0358	0.0807	0.2755	0.0071	0.1828	0.1048	0.175	0.1759	0.4673
TEMP×TIME		<.0001	<.0001	0.2488	0.258	<.0001	0.0936	0.0116	0.0048	0.0009	0.4391

$^{a-i}$ Means within a column with same superscripts are not different at P < 0.05

Table 2.10 (cont.) Effects of changes of time and temperature (TEMP) conditions of heat pretreatment on amino acid (AA) profile, crude protein (CP) content, available Lys content, and Lys to CP ratio of whole stillage.

Factor		***Analyte, g/100 g***											
TEMP, °C	**TIME, min**	**Indispensable AA**								**Total AA**	**CP**	**Avail. Lys**	**Lys:CP**
		Ala	**Asp**	**Cys**	**Glu**	**Gly**	**Pro**	**Ser**	**Tyr**				
0	0	1.85	1.75$^{a\text{-}f}$	0.59^{abcd}	3.65	1.12	2.11$^{a\text{-}j}$	1.21$^{a\text{-}k}$	0.94	28.27	28.27	0.90^{abc}	3.47^{abc}
100	10	1.86	1.78^{a}	0.60^{a}	3.65	1.12	2.13^{a}	1.22^{abe}	0.98	28.69	28.69	0.88^{c}	3.40^{c}
	20	1.86	1.79^{a}	0.61^{a}	3.65	1.13	2.13^{a}	1.23^{a}	0.99	28.53	28.53	0.92^{b}	3.55^{b}
	30	1.87	1.80^{ab}	0.62^{a}	3.65	1.13	2.13^{abcd}	1.24^{abcd}	0.99	28.36	28.36	0.96^{a}	3.69^{a}
120	10	1.86	1.76^{bc}	0.59^{b}	3.66	1.12	2.12^{abe}	1.22^{abef}	0.98	28.82	28.82	0.81^{d}	3.20^{d}
	20	1.86	1.75^{cde}	0.58^{bc}	3.66	1.12	2.11^{be}	1.22^{be}	0.98	28.67	28.67	0.79^{d}	3.19^{d}
	30	1.86	1.75^{cde}	0.57^{bcd}	3.66	1.12	2.11^{ehi}	1.21^{ehg}	0.98	28.52	28.52	0.77^{de}	3.18^{d}
140	10	1.86	1.74^{d}	0.58^{c}	3.66	1.12	2.11$^{a\text{-}h}$	1.21$^{a\text{-}j}$	0.98	28.95	28.95	0.74^{e}	3.00^{e}
	20	1.86	1.72^{f}	0.55^{e}	3.66	1.11	2.10^{cfh}	1.20^{cgj}	0.98	28.81	28.81	0.66^{f}	2.84^{f}
	30	1.85	1.69^{gh}	0.53^{f}	3.67	1.11	2.08^{fj}	1.19^{fjk}	0.97	28.7	28.7	0.59^{g}	2.68^{g}
160	10	1.86	1.73^{efg}	0.56^{de}	3.66	1.11	2.10$^{a\text{-}h}$	1.21^{a}	0.98	29.08	29.08	0.67^{f}	2.81^{fg}
	20	1.85	1.68^{h}	0.52^{f}	3.67	1.1	2.08^{dij}	1.19^{dhik}	0.97	28.96	28.96	0.54^{h}	2.49^{h}
	30	1.84	1.63^{i}	0.48^{g}	3.67	1.1	2.06^{g}	1.17^{i}	0.96	28.8	28.8	0.40^{i}	2.17^{i}
SEM		0.09	0.07	0.06	0.29	0.04	0.1	0.04	0.05	1.01	1.01	0.07	0.2
P - value													
TEMP		0.5746	<.0001	<.0001	0.8135	0.0181	0.0428	0.0353	0.3744	0.0304	0.0304	<.0001	<.0001
TIME		0.5445	0.0659	0.0029	0.8944	0.7429	0.2674	0.4647	0.7689	0.0596	0.0596	0.0013	0.0234
TEMP×TIME		0.3564	<.0001	<.0001	0.8528	0.0526	0.0388	0.0396	0.0604	0.7084	0.7084	<.0001	<.0001

$^{a\text{-}i}$ Means within a column with same superscripts are not different at P < 0.05. More than 5 letters are simplified by a dash (-) indicating consecutive characters.

Table 2.11. Effects of changes of time and temperature (TEMP) conditions of heat pretreatment on total furans, furfural and Hydroxymethylfurfural (HMF) content of whole stillage.

Factor		***Analyte (mg/kg MS)***		
TEMP, °C	**TIME, min**	**Total Furans**	**Furfural**	**HMF**
0	0	0.49^{h}	0.00^{a}	0.64^{l}
100	10	7.55^{g}	0.13	3.83^{k}
	20	4.12^{g}	0.21	2.91^{jkl}
	30	2.25^{gh}	0.34	2.21^{hjkl}
120	10	21.84^{f}	0.42	7.55^{ij}
	20	17.73^{f}	0.61	7.40^{gi}
	30	14.39^{ef}	0.89	7.25^{efgi}
140	10	63.21^{de}	1.31	14.92^{dfgh}
	20	76.23^{cd}	1.76	18.93^{cdef}
	30	91.93^{cd}	2.36	24.02^{bcd}
160	10	182.94^{c}	4.10	29.55^{ce}
	20	327.80^{b}	5.06	48.57^{b}
	30	587.37^{a}	6.23	79.84^{a}
SEM		2.91	1.34	1.50
P - value				
TEMP		<.0001	<.0001	<.0001
TIME		0.4819	0.0797	0.3187
TEMP×TIME		0.0019	0.6349	0.0216

$^{a\text{-}i}$ Means within a column with same superscripts are not different at $P < 0.05$.

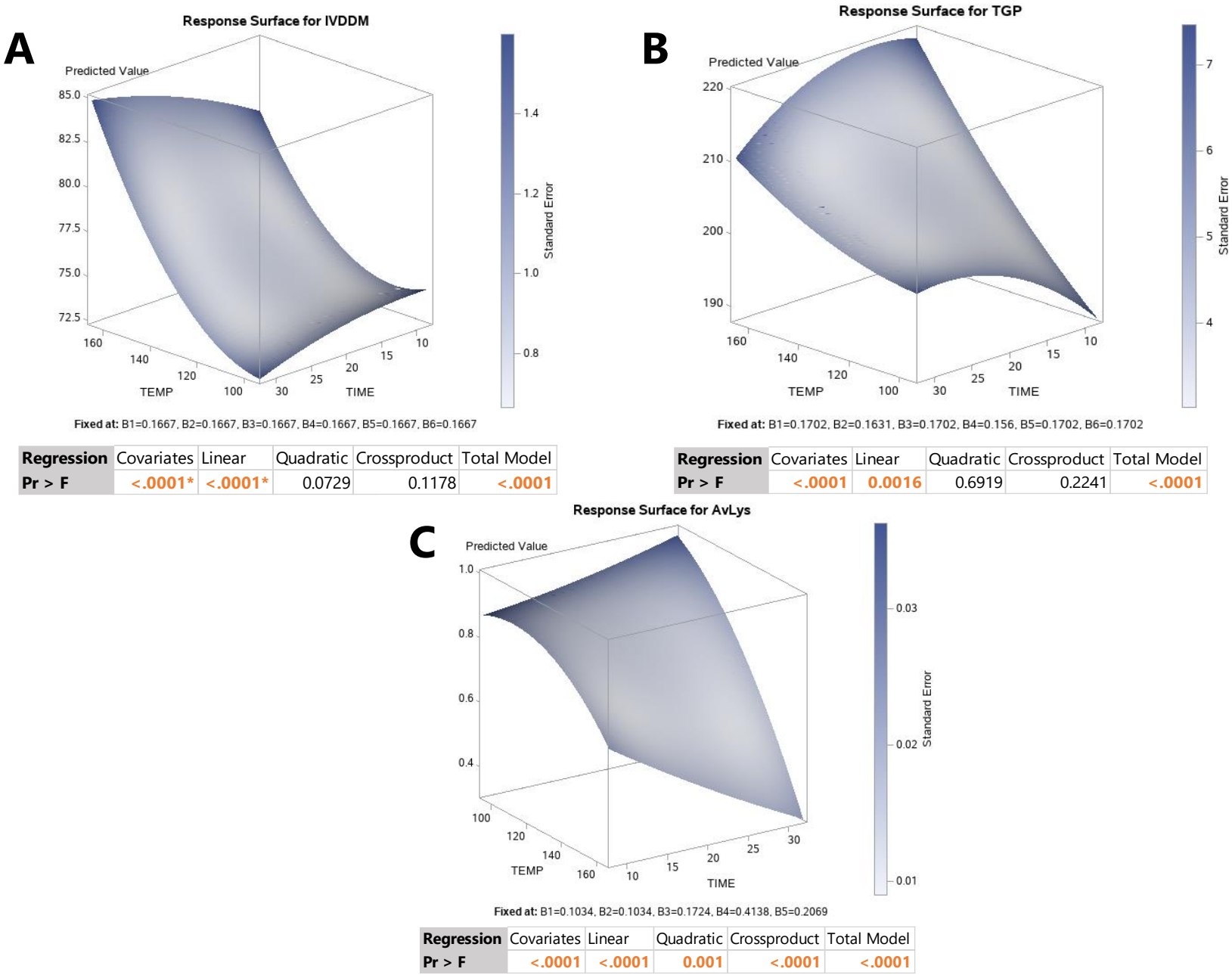
Table 2.12. Effects of changes of time and temperature (TEMP) conditions of heat pretreatment on *in vitro* disappearance (IVD) after pepsin and pancreatin digestion of DM, starch, TDF, IDF and SDF (g/100 g) of whole stillage.

Factor		***Analyte (mg/kg MS)***				
TEMP, °C	**TIME, min**	**IVD-DM**	**IVD-starch**	**IVD-TDF**	**IVD-IDF**	**IVD-SDF**
0	0	73.4	29.42_{fgh}	58.76	57.68	52.11
100	10	73.07	30.89_{g}	57.01	57.72	47.78
	20	74.13	22.71_{h}	56.61	57.76	43.46
	30	73.48	14.53_{i}	56.21	57.36	53.81
120	10	75.71	37.92_{e}	56.93	57.67	49.02
	20	73.94	34.83_{f}	56.72	57.99	44.22
	30	73.51	31.74_{g}	56.51	57.03	55.51
140	10	76.44	44.94_{d}	56.85	57.62	50.25
	20	77.02	46.95_{d}	56.84	58.21	44.99
	30	78.15	48.96_{cd}	56.82	56.7	57.21
160	10	79.27	51.97_{c}	56.78	57.57	51.48
	20	82.48	59.07_{b}	56.95	58.43	45.76
	30	82.73	66.18_{a}	57.12	60.65	45.61
SEM		2.41	4.58	1.54	1.44	6.29
P - value						
TEMP		<.0001	<.0001	0.873	0.631	0.631
TIME		0.608	0.016	0.786	0.239	0.239
TEMP×TIME		0.428	<.0001	0.565	0.789	0.789

[a-i] Means within a column with same superscripts are not different at $P < 0.05$.

Table 2.13. Effects of changes of time and temperature (TEMP) conditions of heat pretreatment of whole stillage on *in vitro* fermentation kinetics parameters obtained from modeling curves of the treatments.

Factor		***Parameter***			
TEMP, °C	**TIME, min**	**Lag Time, h**	**Half Time, h**	**Degradation Rate, g DM/h**	**TGP, mL/g DM**
0	0	3.67	24.01^{abc}	0.046^{efg}	183.92
100	10	3.17	24.34^{a}	0.044^{g}	199.19
	20	3.09	24.83^{a}	0.043^{g}	198.24
	30	3	25.32^{a}	0.041^{g}	197.28
120	10	3.2	23.52^{b}	0.047^{f}	203.15
	20	3.22	23.21^{bc}	0.049^{ef}	202.92
	30	3.24	22.91^{bcd}	0.05^{def}	202.68
140	10	3.22	22.69^{c}	0.05^{e}	207.11
	20	3.35	21.6^{e}	0.055^{c}	207.59
	30	3.47	20.51^{f}	0.059^{b}	208.07
160	10	3.25	21.87^{de}	0.053^{cd}	211.08
	20	3.48	19.99^{f}	0.061^{b}	212.27
	30	3.71	18.11^{g}	0.069^{a}	213.47
SEM		3.31	0.91	0.003	7.23
P-VALUE					
TEMP		0.2313	<.0001	<.0001	0.0007
TIME		0.9103	0.2803	0.0665	0.9017
TEMP×TIME		0.0837	<.0001	<.0001	0.3989



Regression	Covariates	Linear	Quadratic	Crossproduct	Total Model
Pr > F	<.0001*	<.0001*	0.0729	0.1178	<.0001

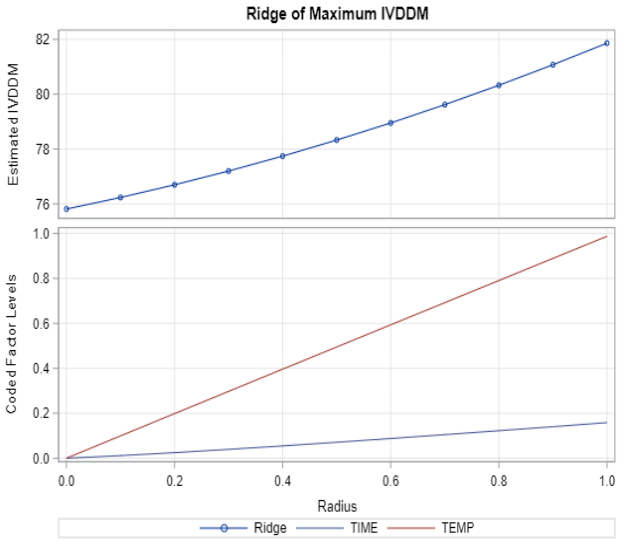
Regression	Covariates	Linear	Quadratic	Crossproduct	Total Model
Pr > F	<.0001	0.0016	0.6919	0.2241	<.0001

Regression	Covariates	Linear	Quadratic	Crossproduct	Total Model
Pr > F	<.0001	<.0001	0.001	<.0001	<.0001

Figure 2.5. Response surface plot and model significance for IVD-DM (A), TGP (B) and available lysine (C; Av Lys) of whole stillage

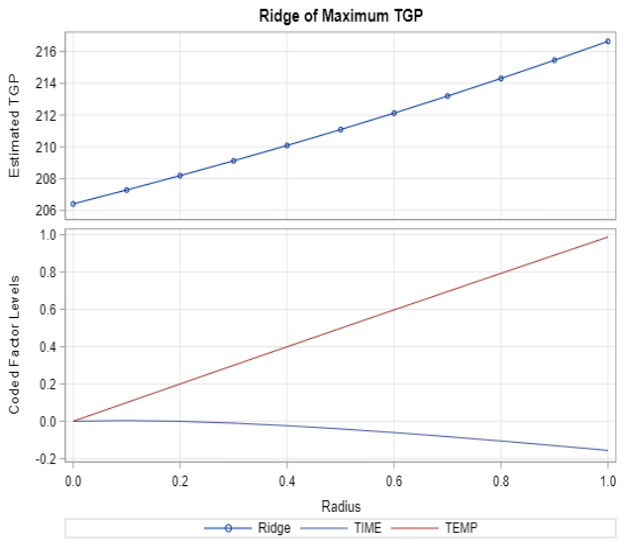
A

Estimated Ridge of Maximum Response for Variable IVDDM				
Coded Radius	Estimated Response	Standard error	Uncoded Factor TIME	Uncoded Factor TEMP
0	75.81	0.81	20.00	130.00
0.1	76.23	0.80	20.12	132.98
0.2	76.69	0.79	20.25	135.95
0.3	77.20	0.78	20.39	138.92
0.4	77.74	0.76	20.55	141.89
0.5	78.33	0.75	20.71	144.85
0.6	78.95	0.74	20.88	147.81
0.7	79.62	0.74	21.05	150.76
0.8	80.32	0.76	21.22	153.72
0.9	81.07	0.81	21.40	156.67
1	81.86	0.89	21.58	159.62



B

Estimated Ridge of Maximum Response for Variable TGP				
Coded Radius	Estimated Response	Standard Error	Uncoded Factor TIME	Uncoded Factor TEMP
0	206.41	3.66	20.00	130.00
0.1	207.28	3.65	20.03	133.00
0.2	208.19	3.60	20.00	136.00
0.3	209.12	3.54	19.90	139.00
0.4	210.09	3.46	19.76	141.98
0.5	211.09	3.39	19.59	144.95
0.6	212.12	3.35	19.39	147.91
0.7	213.20	3.37	19.18	150.85
0.8	214.31	3.49	18.94	153.79
0.9	215.46	3.72	18.69	156.71
1	216.64	4.08	18.44	159.63



C

Estimated Ridge of Maximum Response for Variable AvLys: Available Lysine %				
Coded Radius	Estimated Response	Standard Error	Uncoded Factor TIME	Uncoded Factor TEMP
0	0.79071	0.01301	20	130
0.1	0.80877	0.01318	19.7506	127.095
0.2	0.8252	0.01331	19.5456	124.157
0.3	0.84001	0.01346	19.4071	121.178
0.4	0.85326	0.0137	19.3714	118.149
0.5	0.86506	0.01412	19.4966	115.076
0.6	0.87557	0.01481	19.8595	112.005
0.7	0.88511	0.01581	20.5099	109.056
0.8	0.89407	0.01708	21.4016	106.371
0.9	0.90282	0.01857	22.4242	103.998
1	0.91161	0.02025	23.4927	101.889

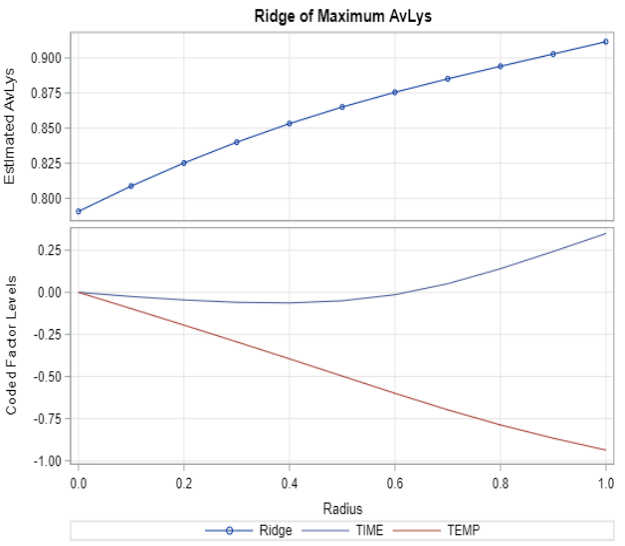


Figure 2.6. Ridge analysis and plot of optimun yields of IVD-DM (A), TGP (B) and Available Lys (C) of whole stillage

3. PORCINE *IN VITRO* DEGRADATION CHARACTERISTICS OF ENZYME PREDIGESTED AND HEAT PRETREATED CORN WHOLE STILLAGE

ABSTRACT. Effects of heat pretreatment (HT) and multi-enzyme predigestion (MP) of whole stillage (WS; slurry material that is dried into DDGS) on porcine *in vitro* digestibility of DM (IVD-DM) and fermentation characteristics of WS were investigated. A sample of WS was obtained from 4 different sources. Half amount of WS from each source was pretreated at 70 psi and 160°C for 20 min. Untreated and heat pretreated WS samples from each source were divided into 4 sub-samples (4 sub-samples of untreated WS per source and 4 sub-samples of pretreated WS per source) to give 32 sub-samples. Four treatments were applied to 32 sub-samples WS (1 untreated or 1 pretreated sub-sample per treatment per sample source). The treatments were WS undigested or pre-digested with 1 of 3 multi-enzymes (MTE1, MTE2, and MTE3). The MTE1 contained xylanase, β-glucanase, cellulase, mannanase, protease and amylase; MTE2 contained xylanase, α-galactosidase, and celullase; and MTE3 contained xylanase, cellulase, β-glucanase and mannanase. The 32 sub-samples were subjected to porcine *in vitro* digestion in 3 cycles of 2 batches (16 sub-samples/batch). Subsequently, residues were subjected to porcine *in vitro* fermentation for 72 hours, during which accumulated gas production was recorded and modeled to estimate kinetics of gas production. The IVD-DM of untreated WS was 73.4%. The HT improved ($P < 0.05$) IVD-DM of WS by 8.2 percentage points. The MP improved IVD-DM of untreated WS and heat-pretreated WS by a means 9.1 and 6.8 percentage points, respectively. However, the magnitude of improvement in IVD-DM of pretreated WS due to predigestion was lower ($P < 0.05$) for MTE3 than that for MTE2 (4.8 vs. 9.0 percentage points), but similar to that for MTE1 (6.7 percentage points). Similar interactions were observed for total gas production. In conclusion, the digestibility of WS was improved by

the HT and MP. Combination of HT and MTE2 predigestion was the most effective in improving digestibility of WS.

3.1. INTRODUCTION

The production of bioethanol from corn has steadily grown over the past 15 years with two major relevant consequences concerning the animal feed industry; increase in competition of corn grain use as a feedstuff and a growing availability of co-products from corn bioethanol industry. Corn DDGS is the most important coproduct of the bioethanol industry; it is used as a source of protein and energy for pigs and poultry. However, corn DDGS has high content of NSP, which negatively affect the digestibility of energy and nutrients in monogastric animals (Stein and Shurson, 2009; Shurson, 2017).

Non starch polysaccharides degrading enzymes, often known as NSPase, have been broadly evaluated for use in swine and poultry diets containing DDGS (Swiatkiewicz et al., 2016). Arabinoxylans are the major NSP component in DDGS (Pedersen et al., 2014; Jaworski et al., 2015). Hence, xylanase alone or in combination with other enzymes such as cellulase, β-glucanase, mannanase, pectinase, galactanase, and invertase have been added to DDGS-based diets for pigs and poultry (Swiatkiewicz et al., 2016). Notwithstanding, the effectiveness of enzyme supplementation to DDGS-based diets for swine and poultry has been inconsistent in the available literature (Swiatkiewicz et al., 2016; Bedford, 2018). The inconsistency has been attributed to several factors including recalcitrance of fibers to enzymatic hydrolysis, the short retention time in the gastrointestinal tract, and the fact that conditions in the gastrointestinal tract often do not allow for maximal enzyme activity (Bedford and Schulze, 1998). Thus, there is a critical

need to develop technologies that increase nutrient availability of DDGS to increase utilization of DDGS in formulating pig and poultry diets.

One efficient approach is to pretreat DDGS before blending into the final ration as pretreatment can increase the susceptibility of fiber to digestion or fermentation in the gastrointestinal tract (see Chapter 2). Another more efficient approach is to enzymatically hydrolyze the DDGS under optimal conditions before blending into the final ration. Indeed, predigestion of untreated or heat-pretreated WS with fiber-degrading enzymes increased porcine *in vitro* digestibility of the WS (Zangaro et al., 2018). As previously mentioned, (see Chapter 2), pretreatment or predigestion of WS can be good technologies of improving the nutritive value of the resulting DDGS because these technologies can be integrated in ethanol plants to minimize the cost of pretreatment or predigestion. Furthermore, in situ predigestion would provide precise control over conditions of predigestion in order to optimize enzyme response. However, information is lacking on optimal time and best enzyme complex for predigesting the WS. The objective of this study was to identify best enzymes complex and incubation period for predigestion of WS for pigs using porcine *in vitro* digestion and fermentation techniques.

3.2. MATERIALS AND METHODS

This study was conducted in two experiments; Experiment 1 was conducted to determine the effects of period of predigesting WS with multi-enzyme and composition of the multi-enzyme on porcine *in vitro* digestibility of dry matter (IVD-DM) of the WS; whereas Experiment 2 investigated the effects of heat pretreatment (HT) and multi-enzyme predigestion (MPD) of WS (for the optimal period of time identified in Experiment 1) on porcine *in vitro* digestibility of DM (IVD-DM) and fermentation characteristics of WS.

3.2.1. Experiment 1

3.2.1.1. Sample Source and Experimental Design. Samples of WS from 4 different sources were freeze-dried and divided into 13 subsamples to give a total of 52 sub-samples. Thirteen treatments were randomly applied to the 48 sub-samples within source. The treatments were undigested WS (control); or pre-digested with 1 of 3 multi-enzymes (MTE1, MTE2, and MTE3) at 55 °C for 6, 12, 18 or 24 h in 3 × 4 factorial arrangement. The MTE1 contained xylanase, β-glucanase, cellulase, mannanase, protease and amylase; MTE2 contained xylanase, α-galactosidase, and celullase; and MTE3 contained xylanase, cellulase, β-glucanase and mannanase.

3.2.1.2. Multienzyme Composition. Enzymes were obtained from three different manufacturers and contained activities of different enzymes. Enzyme crude protein content was quantified as %N × 6.25 using a rapid MAX N exceed apparatus (Elementar Analysensysteme GmbH, Germany). Enzymes dosages, and predigestion temperature and pH were as per manufacturer advise. Information on test enzymes, crude protein content, and prediction temperatures and pH are presented in Table 3.14.

3.2.1.3. Enzymatic Predigestion. Four grams of freeze-dried WS were placed in autoclaved Erlenmeyer flasks, and distilled water was added into the flasks to achieve 10% solid loading rate. The pH was adjusted every two hours with 3.6M H2SO4 or 6M NaOH to the average of the optimal level for each enzyme combination when necessary. Solid enzymes were dissolved in distilled water before application. Following application of enzymes, the flasks were incubated at constant agitation speed of 200 rpm. At 6, 12, 18 or 24 h, a random flask from each combination was withdrawn from the incubator and subjected to porcine in vitro digestion.

3.2.1.4. In Vitro Digestion. Following the predigestion the samples from Experiment 1 were subjected to porcine *in vitro* digestion with porcine pepsin and pancreatin as described by Woyengo et al. (2016). A phosphate buffer solution (200 mL, 0.1 M, pH 6.0), HCl solution (80 mL, 0.2 M) and fresh pepsin (4 mL, 20 g/L porcine pepsin, P-0609; Sigma-Aldrich Corp., St. Louis, MO, USA) were then added into the flasks with the samples. Additionally, 2 mL of chloramphenicol (C-0378; Sigma-Aldrich Corp., St. Louis, MO, USA) solution (0.5 g/100 mL) was added in the flasks to prevent bacterial growth during the enzymatic hydrolysis. The samples were then placed into a water bath at 39 °C for 2 h under a gentle agitation (50 rpm). After pepsin hydrolysis, phosphate buffer solution (80 mL, 0.2 M, pH 6.8), NaOH (20 mL, 0.6 M), and fresh pancreatin solution (8 mL, 100 g/L pancreatin; P-1750 Sigma-Aldrich Corp., St. Louis, MO, USA) were added into the flasks, and digestion was continued for 4 h in water bath at the same conditions under which the samples were digested with pepsin. The residues of the samples after the digestion were collected by filtration on a nylon cloth (50 μm), and then washed with ethanol (2 × 25 mL 95% ethanol) and acetone (2 × 25 mL 99.5% acetone). The washed residues were dried for 12 h at 60 °C and weighed for determination of IVD-DM.

3.2.2. Experiment 2

3.2.2.1. Sample Source and Experimental Design. Four WS samples were obtained from 4 different sources. Half amount of WS from each source was pretreated with heat 70 psi and 160°C for 20 min. Subsequently, untreated, and pretreated WS samples from each source were freeze-dried and divided into 4 sub-samples (4 sub-samples of untreated WS per source and 4 sub-samples of pretreated WS per source) to give 32 sub-samples. Four enzyme treatments were applied to 32 sub-samples WS (1 untreated or 1

pretreated sub-sample per treatment per sample source). The treatments were WS (untreated or heat pretreated) undigested or pre-digested with multi-enzymes used in experiment 1.

3.2.2.2. Heat Pretreatment. The samples were heat pretreated at the National Center for Agricultural Utilization Research (NCAUR) in Peoria, IL. Briefly; 500 ml of whole stillage was added to a 500 ml working volume stainless steel reactor (2" diameter sanitary tubing with fluoroelastomer rubber gaskets, end caps, and bolted high pressure sanitary clamps) then placed in a Techne Industrial Fluidized Sand Bath (model IFB-101, Techne Incorporated, Burlington, NJ). Reactor temperature was monitored using an internal thermocouple probe and brought to 160 °C then held at the target temperature 20 minutes. Reactor was immediately cooled by transferring it to a vessel containing cold water. The pretreated WS was then transferred to individual Nalgene bottles and frozen and shipped to the Department of Animal Science at the South Dakota State University.

3.2.2.3. Enzymatic Predigestion. Untreated and heat pretreated WS were predigested as describe in Experiment 1, except that all flasks were incubated at constant agitation during 12 h, when target time was achieved the samples were withdrawn from the incubator and subjected to porcine *in vitro* digestion.

3.2.2.4. Porcine In Vitro Digestion. Untreated, predigested and heat pretreated WS samples were subjected to porcine *in vitro* digestion as described in Experiment 1. The 32 sub-samples were subjected to porcine *in vitro* digestion in 3 cycles of 2 batches (16 sub-samples/batch).

3.2.2.5. Porcine In Vitro Microbial Fermentation. Fermentation of undigested residues from the *in vitro* enzymatic digestion of predigested untreated or

pretreated WS was evaluated *in vitro* using a cumulative gas-production technique that has been adapted to the pig (Bindelle et al., 2007; Jha et al., 2015). Two hundred milligrams of the undigested residues were weighed into 125 mL-glass bottle (ThermoFischer Scientific, Waltham, MA, USA) containing 30 mL buffer solution that contained macro- and micro-minerals (Menke, 1988) and a freshly prepared pig fecal inoculum. The undigested residues were then incubated in a water bath for 72 h at 39 °C with a slight agitation of 50 rpm.

The feces for preparation of inoculum were collected from the rectum of three growing pigs housed at the Animal Science Complex of South Dakota State University and fed a standard commercial diet with no antibiotics. The collected fecal samples were instantly placed in air-tight plastic syringes to avoid exposure to aerobic conditions. The feces were diluted 20 times in the buffer solution, filtered through a 250 μm-screen sieve, and transferred into bottles with undigested residue. The final concentration of fecal inoculum in buffer solution was 5%. The bottles were completely sealed with rubber stoppers and immediately placed in the water bath for incubation (39 °C). During the preparation of inoculum and its transfer into bottles, anaerobic conditions were maintained by flushing with CO_2 gas. The experimental animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (# 16-069E).

The gas that was generated during fermentation was measured at 0, 2, 5, 8, 12, 18, 24, 36, 48, and 72 h using a pressure transducer (SIN-54978; GP:50, Grand Island, NY, USA) that was fitted with a digital data tracker (Blue Ribbon Corp., Grand Island, NY, USA). The bottles were vented after each reading using a needle. After 72 h of incubation, fermentation was stopped by placing the bottles in ice. The contents of the bottles were

collected and stored in a −20 °C freezer. The experimental scheme for *in vitro* fermentation was as follows: two batches of in-vitro fermentation were conducted, where each batch contained the complete set of triplicated samples from two randomly selected ethanol plants (2×8×3), accompanied by 3 bottles containing only the reagents to serve as sample blanks and three bottles containing inulin as a control for the fermentation, to give a total of 54 bottles per fermentation batch.

3.2.3. Calculations

The value for *in vitro* disappearance of DM after pepsin and pancreatin hydrolysis (IVD-DM, g/100 g) was calculated as follows:

$$IVD_{(g/100g)} = \frac{\text{weight of intact sample} - \text{weight of residue}}{\text{weight intact sample}} \times 100$$

Gas pressure measurements were converted into gas volume (G, per gram DM) using the ideal gas law, assuming an atmospheric pressure of 101,325 Pa and a temperature of 312.15 K. Gas accumulation curves recorded during the 72 h of fermentation were modelled according to France et al. (1993):

$$G\ (mL\ g^{-1}\ DM) = 0, if\ 0 < t < L$$

$$G\ (mL\ g^{-1}\ DM) = G_f\left(1 - \exp\{-(b(t-L) + c\left(\sqrt{t} - \sqrt{L}\right)\}, if\ t \geq L\right.$$

where, G denotes the gas accumulation to time, G_f (mL/g DM) the maximum gas volume for t = ∞ and L (hours, h) the lag time before the fermentation starts. The constants b (h−1) and c (h-1/2) determine the fractional rate of degradation of the substrate μ (h−1), which is postulated to vary with time as follows:

$$\mu = b + \frac{c}{2\sqrt{t}}, t \geq L$$

Kinetics parameters (Gf, L, μt=T/2 and T/2) were compared in the statistical analysis. The T/2 is the time to half-asymptote when G=Gf/2.

3.2.4. Statistical Analyses

The IVD-DM, TGP and fermentation kinetics parameters were subjected to ANOVA using MIXED procedure of SAS (SAS Studio, SAS Institute Inc., Cary, NC). Treatment least square means were separated by the least significant difference. To test the hypotheses, $P < 0.05$ was considered significant. Model residuals were tested for homogeneity and normality.

In Experiment 1, multienzyme treatment and incubation period were included as fixed effects and sample source as random effect. The least square means by multi-enzyme treatment were modeled applying a nonlinear model using the Fit Curve feature of JMP (JMP PRO 14.3.0, SAS Institute Inc., Cary, NC). A Parallelism Test was conducted for testing if the fitted models between MTE groups have the same shape but are shifted along the X axis.

In Experiment 2, multienzyme treatment and heat pretreatment as fixed effects, while WS sample source was included as random effect in the models. In addition to all pairwise comparison a multiple comparison of least squared means was conducted using the Dunnett-Hsu approximation for factor-analytic covariance with the least square mean of untreated WS as the control level.

3.3. RESULTS

The results from Experiment 1 are presented in Table 3.15 and Figure 3.7. The IVD-DM for multi-enzyme undigested WS (control) was not affected by incubation period. Multienzyme supplementation and incubation period interacted ($P < 0.001$) on IVD-DM.

The IVD-DM of the MTE predigested WS increased ($P < 0.05$) with increase in incubation period. However, an increase in the incubation period from 0 to 12 h resulted in a greater ($P < 0.05$) change in the mean IVD-DM than an increase in the incubation period from 12 to 24 h. Incubation period and multi-enzyme type interacted on IVD-DM such that the magnitude of improvement in IVD-DM between 0 and 12 of predigestion differed ($P < 0.05$) among the 3 multi-enzyme types. The curves of IVD-DM against incubation period for the tree types of MTE were modeled (Figure 3.7). The model with the lower Akaike information criterion corresponded to a 4-parameter logistic curve and the model R^2 was 0.99. The parallelism test was significant ($P < 0.05$), indicating that the group models are significantly different from one another. The estimated maximum response of IVD-DM for MTE1, MTE2 and MTE3 were 82.8%, 85.1% and 87.5% at 19.2, 15.8 and 15.3 h, respectively.

The effects of WS predigestion and heat pretreatment on IVD-DM are presented in Table 3.16. Heat pretreatment or predigestion of WS increased ($P < 0.05$) IVD-DM of untreated WS. Heat pretreatment and predigestion of WS interacted ($P < 0.001$) on IVD-DM such that the magnitude of improvement in IVD-DM of untreated WS due to multi-enzyme predigestion was not affected by multi-enzyme type; however, the magnitude of improvement in IVD-DM of for heat pretreated WS due to multi-enzyme predigestion was lower for MTE3 than for MTE1 or MTE2.

Table 3.17 shows the fitted *in vitro* fermentation parameters of WS and the effects of multienzyme predigestion and heat pretreatment. Heat pretreatment or predigestion of WS increased ($P < 0.05$) total gas production of untreated WS. Heat pretreatment and predigestion of WS interacted ($P < 0.001$) on TGP such that the magnitude of improvement

in TGP for untreated WS due to multi-enzyme predigestion was not affected by multi-enzyme type; however, the magnitude of improvement in TGP of for heat pretreated WS due to multi-enzyme predigestion was lower for MTE3 than for MTE1 or MTE2. Heat pretreatment and predigestion of WS did not interact ($P > 0.05$) on lag time, half time to asymptote and degradation rate. Heat pretreatment increased ($P < 0.05$) lag time and half time to asymptote for undigested residue of WS. Predigestion or heat treatment increased ($P < 0.001$) degradation rate of in-vitro-undigested residue of WS.

3.4. DISCUSSION

The objective of this study was to evaluate the effects of heat pretreatment and subsequent multienzyme predigestion of corn WS on porcine *in vitro* characteristics. Experiment 1 was designed to establish the optimal incubation period for WS with the multi-enzyme products used in this study. The IVD-DM was affected by the type of MTE used in this study and the optimum period of incubation with multi-enzyme were identified to be 19.2, 15.8 and 15.3 h for MTE1, MTE2 and MTE3, respectively. The increased IVD-DM is consistent with the results from the study of Zangaro et al. (2018) who observed increased *in vitro* the digestibility of WS due to predigestion of the WS with multi-enzyme. The porcine *in vitro* digestion technique measures the disappearance of test feedstuff or feed components (i.e., the amount of the components that are solubilized). The solubilization of feedstuff or feed components is related to their in vivo degradation (de Vries et al., 2013). The WS has high content of NSP, and multi-enzyme products used in this study can potentially degrade NSP. The NSP can reduce accessibility of digestive enzymes to their substrate (de Vries et al., 2012; de Vries et al., 2013). Christensen et al. (2007), reported increased porcine IVD-DM and reduced insoluble NSP fraction of liquid

feed incubated with NSPase, in comparison with the control based on the original dry feed. Therefore, the increase in IVD-DM due to multienzyme predigestion might have been due to increased accessibility of nutrients for porcine *in vitro* degradation and increased solubilization of NSP components by the multienzymes. Christensen et al. (2007), reported that 8 h of incubation were necessary to achieve steady solubilization of NSP and DM in liquid feed incubated with NSPase. In the current study longer periods of incubation were necessary in order to achieve and steady solubilization of DM. Inversely, Choct et al. (2004), observed negative impact of xylanase addition to liquid feed fermented during 1 h on energy digestibility in weaned pigs and no effect when the feed was fermented during 15 h. Determination of an optimum incubation period is important in order to avoid excessive losses of OM or proliferation of harmful microorganism, which in turn might lead to loss of nutritional value of the feedstuff (Choct et al., 2004; Canibe and Jensen, 2012).

Experiment 2 determined the effects of multi-enzyme predigestion and heat on porcine *in vitro* characteristics with the goal of identifying the best multienzyme complex for predigestion of the WS. The IVD-DM of WS was increased by predigestion or pretreatment, which was likely due to degradation of NSP, leading to increased NSP solubilization and availability NSP-encapsulated nutrients for digestion by gastric and pancreatic enzymes by the predigestion or pretreatment. The IVD-DM values of multi-enzyme predigested and heat-pretreated WS in this study were lower than those reported by Zangaro et al. (2018), which could be explained differences in sources of WS used among these studies. The WS used in the study of Zangaro et al. (2018) was obtained from one source, whereas WS used in the current was obtained from 4 different ethanol plants.

Digestibility of DGGS vary depending of its source (ethanol plant; Pahm et al. (2008); Stein et al. (2009), implying that the digestibility of WS can also vary depending on its source. In the current study the multi-enzymes treatments had similar effects on IVD-DM of untreated WS, but not of heat pretreated WS. Various enzymes can act additively or synergistically with regard to hydrolysis of lignocellulosic material (Van Dyk and Pletschke, 2012). Since the multienzymes treatments utilized in this experiment were heterogeneous, the effect of multi-enzyme predigestion on IVD-DM of WS was expected to vary with type of multi-enzyme. Experiment 1 results indicated that the 3 multi-enzyme products used in this study have different incubation periods for optimum IVD-DM for WS. In Experiment 2, the incubation period was 12 h for all the multi-enzyme treatments. Therefore, the lack of differences in treatment with multienzymes on untreated WS may be attributed to the fact that the incubation period was not enough for detection of differences among the enzymes. Pretreatment and enzymatic hydrolysis can act additively in enhancing the degradation of lignocellulosic material (Zhang and Lynd, 2004; Van Dyk and Pletschke, 2012), which is consistent with observations of the Experiment 2 in which IVD-DM of WS was improved by heat pretreatment and multi-enzyme predigestion of heat-pretreated WS resulted in a further increase in IVD-DM. The mechanisms by which pretreatment can enhance enzymatic hydrolysis of lignocellulosic biomass include: degradation of hemicellulose that hampers access of cellulases to cellulose; disruption of the hemicellulose structure; reduction in crystallinity of the cellulose; disruption of the lignin structure and its linkages with the various components of lignocellulose mass (Van Dyk and Pletschke, 2012). As mentioned earlier, the multi-enzyme treatments had dissimilar effects on IVD-DM of pretreated WS, which can be explained by differences in

additivity or synergism of enzymes in the multi-enzyme products. Xylanase and cellulase acted synergistically in degradation of corn cell wall (Murashima et al., 2003). Differences in the rates of hydrolysis of insoluble wheat flour arabinoxylan by different xylanases has been reported in the past (McCleary et al., 2015), implying the efficacy of the enzymes can vary depending on their source (i.e., microorganisms or plants that are used to produce the enzymes). Thus, the differences among the 3 multi-enzyme products with regard to IVD-DM of pretreated WS could attributed to differences in sources of the multi-enzymes. The differences could also have been due to the different composition of the enzyme complexes used in the current study. The most abundant NSP in corn DDGS are cellulose or arabinoxylans, which constitute ~70% of the total NSP composition (Jaworski et al., 2015). Therefore, cellulase and xylanases are considered to be the core enzymes for NSP degradation of this biomass. Nevertheless, for arabinoxylans, the presence of arabinose substitutions requires a host of ancillary enzymes to remove branches from the xylan backbone to give access to the core enzymes to degrade the xylan backbone (Van Dyk and Pletschke, 2012). For instance, α-galactosidase and mannanase have shown to act synergistically in enhancing accessibility of xylanase to its subtract (Clarke et al., 2000; Visser et al., 2013). The MTE2 product used in the current study contained α-galactosidase as accessory enzyme. Furthermore, corn DDGS has been reported to have a greater content of mannose as a proportion of both soluble and insoluble non cellulosic polysaccharides (Pedersen et al., 2014; Jaworski et al., 2015), which can be attributable to the presence of mannans coming from yeast. The MTE1 and MTE3 products in the current study contained mannanase as accessory enzyme. The differences in the magnitudes of improvement in IVD-DM of pretreated WS, but not of untreated WS due to predigestion with different

multi-enzymes could be attributed to the fact that the pretreatment increased the availability of substrates for most multi-enzymes, and hence the effects of multi-enzymes on IVD-DM of WS was less confounded by differences in substrate availability for the multi-enzymes.

Heat pretreatment or multi-enzyme predigestion of WS increased TGP of untreated WS. However, de Vries et al. (2013) did not observe any effects of processing or NSPase predigestion of corn grain and DDGS on total gas production. The processing technologies that were used in the study of de Vries et al. (2013) were wet-milling, extrusion, autoclaving and pretreatment with maleic acid at low concentration. The NSPase product that was used in the study of de Vries et al. (2013) contained endo-1,4-β-xylanase and endo-1,4-β-glucanase, and was directly added to corn grain and DDGS during first incubation step of the *in vitro* digestion process, implying that there was limited time of interaction between the NSPase and NSP in corn and DDGS. Thus, the differences between the results from the current study and that of de Vries et al. (2013) with regard to total gas production could partly be attributed to differences in processing technologies used, composition of NSPase products used, and stage of adding the NSPase products to the fibrous feedstuffs. Bindelle et al. (2011) observed improvement in porcine *in vitro* fermentation kinetics and total gas production of wheat due its predigestion with xylanase and β-glucanase. Furthermore, hydrothermal pretreatment has been used in the past to improve biogas production from lignocellulosic materials (Chandra et al., 2012a; Chandra et al., 2012b; Papa et al., 2015), implying that it increases fermentability of lignocellulose materials. Cellulose compared with non-cellulose NSP like arabinoxylans is poorly fermented due to its crystalline form (Jaworski et al., 2015). Thus, heat pretreatment can increase cellulose fermentation by disrupting its structure, leading to its increased

availability to microorganisms for fermentation. Furthermore, oligosaccharides and other short fragments that are generated from NSP during heat pretreatment or multi-enzyme predigestion are more fermentable than complex polysaccharides structures (Tiwari et al., 2019). Therefore, the enhanced TGP of WS due to heat pretreatment or multi-enzyme predigestion is the result of the increased generation of readily fermentable dietary fiber components by the pretreatment and predigestion.

The magnitude of improvement in TPG due multi-enzyme predigestion was greater for heat pretreated WS than for untreated WS. As previously mentioned, heat pretreatment can disrupt the structure of fiber, leading to increased susceptibility of fiber to multi-enzymatic degradation. The multi-enzymes can then degrade the highly susceptible fiber into fragments that are highly fermentable. Thus, the greater magnitude of improvement in TPG for heat pretreated WS than for untreated WS due to multi-enzyme predigestion could be attributed to increased susceptibility of fiber (to enzymatic degradation) in heat pretreated WS. Similarly, hydrothermal pretreatment enhanced enzyme action on lignocellulosic biomass with regard to biogas production (Hosseini-Koupaie et al., 2019), implying that hydrothermal pretreatment increased the susceptibility of fiber in lignocellulose mass to degradation by the enzymes into fragments that were highly fermentable. In the current study the multi-enzymes did not differ with regard to their effects on TGP for untreated WS, but differed with regard to their effects on TGP for heat pretreated WS, which as previously for IVD-DM, could have been due to greater availability of substrates for most multi-enzymes digestion for heat pretreated WS than for untreated WS.

3.5. CONCLUSION

An increase in multi-enzyme predigestion period from 0 to 24 resulted in increased in IVD-DM. The estimated maximum response of IVD-DM for MTE1, MTE2 and MTE 3 were 82.4, 84.7 and 87.1 g/100g at 15.8, 13 and 13.1 h, respectively. Predigestion of WS with MTE products used in the current study increased porcine *in vitro* digestibility and fermentation of the untreated and pretreated WS. The magnitude of improvement in IVD-DM due multi-enzyme predigestion of untreated WS was similar to the magnitude of improvement in IVD-DM due multi-enzyme predigestion of heat pretreated WS. However, magnitude of improvement in TGP due multi-enzyme predigestion of untreated WS was lower than the magnitude of improvement in TGP due multi-enzyme predigestion of heat pretreated WS. Multienzyme products used in the current study differed with regard to magnitude by which they improved IVD-DM and TGP for heat pretreated WS. Combination of heat pretreatment and MTE2 (xylanase, cellulase, α-galactosidase) predigestion of WS was the most effective on improving *in vitro* digestibility and fermentability of the feedstuff.

3.6. LITERATURE CITED

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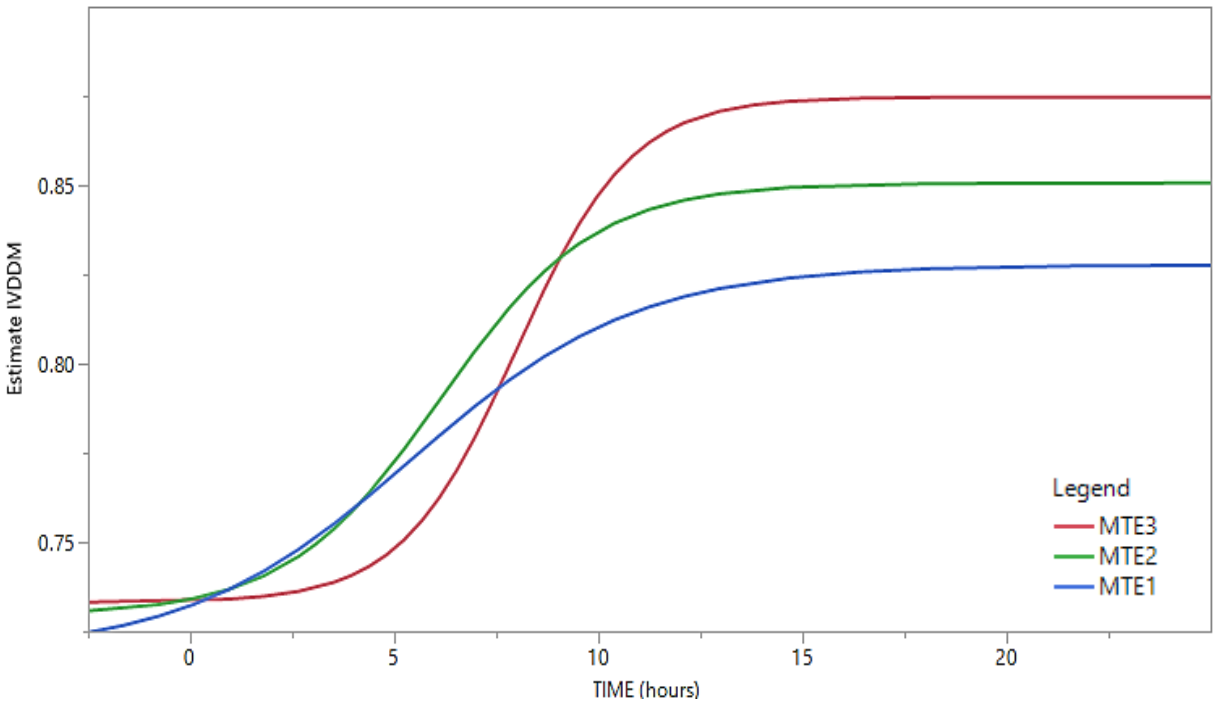
Table 3.14. Multi-enzyme products and ideal conditions according to manufacturer.

Treatment	***Manufacturer***	***Type***	***CP (%)***	***Dosage (mg/g)***	***Optimum temp (°C)***	***Optimum pH***
MTE1	Superzyme	Cocktail	2.24	2.61	38	4.5-5.5
MTE2	BIO-CAT	Xylanase	8.86	2.4	55	4.8
MTE2	BIO-CAT	α-galactosidase	6.03	2.3	55	3
MTE2	BIO-CAT	Celullase	42.52	2	55	5
MTE3	AB Vista	Xylanase	3	2.4	60	5.3
MTE3	AB Vista	β-glucanase	3.533	2.3	60	5.3
MTE3	AB Vista	Mannanase	63.461	2	60	5.3

Table 3.15. Effects of multi-enzyme type (MTE) and predigestion period (PP) on *in vitro* digestibility of dry matter (IVD-DM)

Multi-enzyme treatment	***Predigestion period (Hours)***	***IVD-DM (g/100g)***
Control	**6**	73.36 [j]
	12	73.40 [j]
	18	73.22 [j]
	24	73.17 [j]
MTE1	**6**	76.08 [i]
	12	86.70 [bc]
	18	87.37 [ab]
	24	87.57 [a]
MTE2	**6**	78.81 [h]
	12	84.57 [de]
	18	84.92 [cd]
	24	85.18 [cd]
MTE3	**6**	77.88 [hi]
	12	81.90 [g]
	18	82.45 [fg]
	24	82.92 [ef]
SEM		0.623
***P*-value**		
Multi-enzyme treatment (MTE)		<.0001
Predigestion period		<.0001
MT × PP		<.0001

[abcdefg] Means within a column with similar superscripts are not different at P < 0.05.



AICc	BIC	SSE	MSE	RMSE	R-Square
221.863	-132.932	1.19E-05	3.97E-06	0.001992	0.99969

Group	Parameter	Estimate	SEM	Prob > χ^2	Lower 95%	Upper 95%
MTE1	Growth rate	0.16	0.025	<.0001	0.11	0.21
	Inflection point	5.44	0.489	<.0001	4.48	6.40
	Lower asymptote	0.72	0.007	<.0001	0.71	0.73
	Upper asymptote	0.83	0.002	<.0001	0.82	0.83
MTE2	Growth rate	0.23	0.038	<.0001	0.16	0.31
	Inflection point	6.13	0.168	<.0001	5.80	6.46
	Lower asymptote	0.73	0.003	<.0001	0.72	0.74
	Upper asymptote	0.85	0.001	<.0001	0.85	0.85
MTE3	Growth rate	0.31	0.025	<.0001	0.26	0.36
	Inflection point	7.99	0.195	<.0001	7.61	8.37
	Lower asymptote	0.73	0.002	<.0001	0.73	0.74
	Upper asymptote	0.87	0.001	<.0001	0.87	0.88

Figure 3.7. Curve fit of IVD-DM against predigestion period by multienzyme type and model parameters

Table 3.16. Least square mean values of *in vitro* disappearance of DM (IVD-DM) and effects of multi-enzyme predigestion and heat pretreatment

Predigestion	***Pretreatment***	***IVD-DM (g/100g)***
Control	No	73.40[d]
MTE1	No	81.17[c]
MTE2	No	82.50[c]
MTE3	No	83.79[c]
Control	Yes	81.59[c]
MTE1	Yes	88.25[ab]
MTE2	Yes	90.67[a]
MTE3	Yes	86.38[b]
SEM		1.67
P-value		
Predigestion		0.0002
Pretreatment		<.0001
Predigestion × Pretreatment		<.0001

[abcd] Means within a column with similar superscripts are not different at $P < 0.05$.

Table 3.17. Fitted kinetics parameters of gas accumulation during *in vitro* fermentation of whole stillage and effects of multi-enzyme predigestion and heat pretreatment

Predigestion	*Pretreatment*	*Lag time*	*Degradation rate*	*Half time*	*Total gas*
Control	**No**	3.67^{e}	0.048^{c}	24.96^{bc}	192.17^{e}
MTE1	**No**	3.90^{d}	0.048^{c}	24.26^{c}	199.05^{d}
MTE2	**No**	3.78^{d}	0.050^{c}	25.67^{bc}	203.45^{d}
MTE3	**No**	3.96^{d}	0.050^{c}	25.87^{bc}	201.47^{d}
Control	**Yes**	3.91^{d}	0.050^{c}	24.82^{bc}	202.58^{d}
MTE1	**Yes**	4.07^{b}	0.053^{ab}	28.43^{a}	222.65^{b}
MTE2	**Yes**	4.34^{a}	0.055^{a}	28.73^{a}	227.97^{a}
MTE3	**Yes**	4.11^{c}	0.053^{b}	26.98^{ab}	218.20^{c}
SEM		0.729	0.002	0.868	17.958
P-value					
Predigestion		0.2364	0.001	0.001	<.0001
Pretreatment		0.0107	<.0001	<.0001	<.0001
Predig × Pretreat		0.4736	0.165	0.165	<.0001

^{abcd} Means within a column with similar superscripts are not different at $P < 0.05$.

4. NUTRIENT DIGESTIBILITY OF HEAT PRETREATED OR MULTIENZYME TREATED CORN WHOLE STILLAGE FOR PIGS

ABSTRACT. The use of corn distillers grains with solubles (DDGS) in diets for monogastrics is limited by its high level of dietary fiber and low quality of protein. Pre-treatment of whole stillage (WS; slurry material that is dried into DDGS) with heat or enzymes can improve porcine in vitro digestibility of the resulting DDGS. A study was conducted to determine the apparent ileal digestibility (AID) of gross energy (GE), standardized ileal digestibility (SID) of amino acids (AA) and net energy value (NE) for pigs of heat-pretreated or enzyme predigested corn WS. Ten ileal-cannulated barrows (initial BW =65.6 ± 3.5 kg) were fed 5 diets in a replicated 5 × 5 Latin square design. The diets were cornstarch-based, containing corn DDGS, untreated WS (C-WS), heat-pretreated WS (Heat-WS) or enzyme-predigested WS (Predigested-WS) as sole protein source, and N-free diet. Digestibility of AA in feedstuffs was determined by the direct method. Energy digestibility in feedstuffs was determined by difference from the N-free diet. The WS was heat pretreated at 140 °C and 70 psi for 15 min. Predigestion of the WS was achieved by incubating WS with multienzyme that supplied xylanase, celullase, α-galactosidase at 2.4, 2.0 and 2.3 mg per gram of WS, respectively, for 12 h at 55 °C. On DM basis, DDGS, C-WS, Heat-WS, Predigested-WS contained 32.8, 30.8, 28.18, and 39.7% CP, 39.8, 51.0, 52.2 and 53.8% NDF, and 4.5, 4.6, 5.7 and 4.5% EE, respectively. The AID of GE for C-WS (44.7%) did not differ from that of DDGS (50.1%) and was lower ($P < 0.05$) than that for Predigested-WS by 51%, but greater than that for Heat-WS by 41%. The SID of Lys for C-WS (75.5%) was greater $P < 0.05$) than that for C-DDGS (67.4%) and Heat-WS (53.9%), but lower ($P < 0.05$) than for Predigested-WS (84.1%). The NE value for C-WS (2,793 kcal/kg) did not differ from that of C-DDGS (2,668 kcal/kg

DM). The NE value for C-WS was greater ($P < 0.05$) than that for Heat-WS (1,834 kcal/kg DM) and lower ($P < 0.05$) than that for Predigested-WS (2,814 kcal/kg DM). In conclusion, enzymatic predigestion of WS increased its SID of Lys and NE value, and hence enzymatic predigestion can be an attractive technology to increase the nutritive value of corn DDGS for pigs. Heat pretreatment reduced SID of AA and NE values of the WS, and hence pretreatment of WS at conditions used in the current study may negatively affect its nutritive value.

4.1. INTRODUCTION

Recent trends in the demand and supply of traditional feedstuffs for formulating swine diets have generated a worldwide consideration of low-cost alternatives such as cereal co-products from the biofuels and milling industries to minimize feed costs (Woyengo et al., 2014; Agyekum and Nyachoti, 2017). One of the most commonly used co-product for formulating livestock feed is corn DDGS, which is co-product from the bioethanol industry (Shurson, 2017). Nevertheless, corn DDGS is high in DF, and Maillard reactions might occur during drying stage of its production (Fontaine et al., 2007; Woyengo et al., 2014). Elevated levels of DF negatively affects the energy value of feeds for pigs (Noblet and Le Goff, 2001) and reduces nutrient digestibility (Wenk, 2001), whereas decreased concentration and digestibility of Lys and other amino acid (**AA**) is expected as a result of Maillard reactions that occur during drying stage of producing DDGS (Almeida et al., 2013).

Processing technologies and the use of supplemental enzymes have been proposed to alleviate negative effects of DF or NSP in corn DDGS by increasing its nutrient digestibility and energy value (de Vries et al., 2012; de Vries et al., 2013; Swiatkiewicz et

al., 2016). Furthermore, biorefinery strategies represent a feasible way for upgrading DDGS (Chatzifragkou et al., 2015), and depending on the approach, these strategies might be incorporated into the DDGS production process at relatively low cost (Li et al., 2019). From the available pretreatment technologies, hydrothermal treatment represents an interesting or viable option because it does not: (1) involve use of any chemicals (except water), (2) erode pretreatment equipment, and (3) result in production of substantial amounts compounds that inhibit enzymatic digestion or microbial fermentation (Alvira et al., 2010; Zhuang et al., 2016). Heat pretreatment of WS can be considered as hydrothermal treatment because heat and pressure are applied to the biomass with no need of addition of water because WS is a high-moisture product. Moreover, enzymatic predigestion of biomass particularly with NSP-degrading enzymes is gaining more interest due to the relatively short incubation times, increased availability of commercial enzymes and resistance to enzyme inhibiting compounds (Hosseini-Koupaie et al., 2019). Heat pretreatment and predigestion of WS improved porcine in vitro digestion and fermentation characteristics of the resulted feedstuff (Chapter 2; Chapter 3). However, there is lack of information on the effects of heat pretreatment or enzymatic predigestion of NSP-degrading enzymes on in vivo (pig) nutrient and energy digestibility. The objective of the present study is to determine the effects of heat pretreatment and enzymatic predigestion of WS on nutrient digestibility and energy value of the resulting DDGS for growing pigs.

4.2. MATERIALS AND METHODS

Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (#19-025A).

4.2.1. Experimental Animals

Ten crossbred ileal-cannulated barrows (initial BW of 65.6 ± 3.5 kg; Large White-Landrace female × Large White-Hampshire male; Pig Improvement Company) were used in the study. Pigs had been surgically fitted with a simple T-cannula at the distal ileum as described by Sauer and Ozimek (1986) and used in previous study to determine DE and nutrient profiles of corn DDGS and wheat bran supplemented with xylanase and pectinase. During the 15 days preceding this current study, pigs were fed a grower diet with no additives. Pigs were housed individually in metabolic crates (1.5 × 0.6 × 0.8 m) with smooth polyvinyl chloride walls and plastic-covered expanded metal flooring in a temperature-controlled room (22 ± 2ºC). Each metabolic crate had smooth sides, plastic-covered expanded metal flooring, a single-space dry feeder, and a nipple drinker.

4.2.2. Experimental Diets

Diets included a cornstarch-based diet with conventional DDGS, untreated WS (**C-WS**), heat-pretreated-WS-containing diet (**Heat-WS**), or multienzyme-predigested-WS (**Predigested-WS**); and a N-free diet (Table 4.18). The diets contained titanium dioxide (0.4%) as an indigestible marker. The N-free diet was fed to estimate basal endogenous AA losses for determining standardized ileal digestibility (**SID**) of AA. The test feedstuffs were the sole source of protein in the test diets. The ratio of cornstarch to sugar, cellulose and soybean oil in the test diets was identical to the N-free diet to allow calculation of energy digestibility of the test diets using the difference method (Fan and Sauer, 1995). The corn DDGS and corn WS were kindly provided by the POET Research Center (Scotland, SD). The WS was pretreated at the POET Research Center (Scotland, SD) at 140 °C and 70 psi for 15 min. Untreated and heat pretreated WS were shipped in undried state to Prairie Aquatech (Brookings, SD) where half of the untreated WS was subjected to

predigestion with multi-enzyme for 12 h at 55°C under constant agitation. Untreated WS, heat pretreated WS and predigested WS were dried under mild temperature (<100°C) at Prairie Aquatech (Brookings, SD). The multi-enzyme was obtained from BIOCAT (Troy, VA, USA) and supplied xylanase, celullase, α-galactosidase at 2.4, 2.0 and 2.3 mg per gram of WS, respectively. Following drying untreated WS, heat pretreated WS and predigested WS were ground in a hammer mill to pass through a 2.8 mm screen before their inclusion in diets

4.2.3. Experimental Design and Procedure

The 10 pigs were fed 5 diets in a replicated 5 × 5 Latin square design to give 10 replicates per diet. Each period consisted of 7 d; the first 5 d were for adaptation, followed by 1 d of fecal collection and 1-day ileal digesta collection. Pigs were fed diets at 3 times maintenance energy requirement (3 × 197 kcal of ME/kg of BW^0.6; NRC, 2012) based on BW at the beginning of each period. Daily feed allowance was offered in 2 equal portions at 0900 and 1700 h. Representative fecal samples were collected continuously from each pen for 24 h. Ileal digesta was collected continuously for 24 h from a plastic bag fixed to the canula. The plastic bags contained 5 ml of 10% formic acid to limit microbial growth and were replaced every 30 to 60 minutes. Collected feces and ileal digesta were pooled for each pig and period and stored frozen at -20°C.

4.2.4. Sample Preparation and Analyses

Pooled fecal samples were oven-dried at 60°C for 72 h, whereas pooled ileal digesta samples were freeze-dried for 7 d. Feedstuffs (DDGS, C-WS, Heat-WS and Predigested-WS), diets, dried feces and ileal samples were ground to pass through a 0.75-mm screen using a centrifugal mill (model ZM200; Retsch GmbH, Haan, Germany).

Feedstuffs were analyzed for dry matter (**DM**), organic matter (**OM**), ash, gross energy (**GE**), crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), ether extract (**EE**), and AA. Diets, ileal digesta, and feces were analyzed for DM, GE, CP, and titanium dioxide. Diets and ileal digesta were additionally analyzed for AA. Samples were analyzed for DM (method 930.15), CP (method 984.13A-D), EE (method 920.39A), NDF (method 2002.04), and ADF (method 973.18) according to the AOAC (2012). The GE was analyzed using an adiabatic bomb calorimeter (model 1261, Parr Instrument Co., Moline, IL). Titanium dioxide in samples was determined by spectrophotometry reading the absorbance at 408 nm (Synergy 2, Biotek. Vermont, USA) after ashing at 525°C for 10 h and digestion in concentrated H_2SO_4 (Myers et al., 2004), OM and ash content were obtained from this process. Samples were analyzed for AA (method 975.44; 982.30 AOAC, 2006) at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO). Starch content was determined using the Megazyme Total Starch Assay Kit (Megazyme International Ireland Ltd., Bray Business Park, Bray Co., Wicklow, Ireland).

4.2.5. Calculations and Statistical Analysis

The apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) values of the diets were calculated using the indicator method (Eq. [2]; Stein et al., 2007). Each pig fed the N-free diet was used to calculate its basal endogenous AA losses (Eq. (3); Stein et al., 2007). The SID for AA in diets was calculated from AID corrected for basal endogenous AA loss (Eq. [7]; Stein et al., 2007). The AA digestibility in the test ingredients was determined by the direct method (Fan and Sauer, 1995) and the energy digestibility was determined by the difference method (Fan and Sauer, 1995) from N-free diet. The DE

value of the test ingredient was calculated by multiplying GE by its ATTD. The NE values of feedstuffs (kcal/kg of DM) were predicted from the determined DE (kcal/kg of DM) values and analyzed macronutrient content (g/kg of DM) of feedstuffs using the following equation that was developed by Noblet et al. (1994) and adopted as Eq. (1–18) by NRC (2012):

$$NE = 0.700\,DE + 1.61\,EE + 0.48\,starch - 0.91\,CP - 0.87\,ADF$$

Data were subjected to ANOVA using the MIXED procedure (SAS Inst. Inc., Cary, NC) with the diet as a fixed factor, and pig and period as random factors. Treatment means were separated by probability of difference. To test the hypotheses, the level of significance was set at 5%.

4.3. RESULTS

The analyzed composition of feedstuffs is presented in Table 4.19. The CP, EE, starch and ADF contents of DDGS and C-WS were similar. The NDF content of DDGS was lower than that of C-WS. The EE and NDF contents of C-WS were similar to those of Heat-WS or Predigested-WS, whereas the CP content of C-WS was slightly greater than that of Heat-WS and lower than that of Predigested-WS. The ADF content of C-WS was similar to that of the Predigested-WS, but lower than that of Heat-WS. The starch content of C-WS was higher than that of Heat-WS or Predigested-WS. The levels of indispensable AA (except for Met, Phe and Leu) in DDGS were higher than those of C-WS. The most abundant indispensable AA in DDGS and C-WS were Leu, Phe and Val, whereas Trp, Met and His were the least. The levels of indispensable AA in Heat-WS were lower than those of C-WS. Though the most abundant indispensable AA in Heat-WS were same as those in C-WS, Lys was one of the three least abundant AA in Heat-WS. The levels of indispensable

AA in Predigested-WS were higher to those of C-WS. The most and least abundant indispensable AA in Predigested WS were same as those in C-WS.

The AID and SID of CP and AA of the feedstuffs are presented in Table 4.20 and Table 4.21, respectively. The AID of CP and indispensable AA for DDGS were lower ($P < 0.05$) than those for C-WS. The AID of CP and all indispensable AA for C-WS were greater than those for Heat-WS. The AID of CP and all indispensable AA for C-WS did not differ from those for Predigested-WS. The SID of all indispensable AA for C-WS were greater ($P < 0.05$) than those of DDGS, except for Arg, which did not differ. The SID of indispensable AA for C-WS were greater ($P < 0.05$) than those of Heat-WS. The SID all indispensable AA for C-WS did not differ from those for Predigested-WS, except for Lys, which was lower ($P < 0.05$) in C-WS.

The AID and ATTD of DM, OM, and GE of diets are presented in Table 4.22. The AID of DM, OM, and GE; ATTD of DM, OM, GE, NDF and ADF; and DE and NE values of the feedstuffs are presented in Table 4.23. The AID of GE for DDGS-based diet and DDGS did not differ from that of C-WS based diet and C-WS. The AID of GE for C-WS-based diet and C-WS was greater ($P < 0.05$) than that for Heat-WS, but lower ($P < 0.05$) than that for Predigested-WS-based diet and Predigested-WS. The ATTD of GE for C-WS was greater ($P < 0.05$) than that for DDGS. The ATTD of GE for C-WS was greater ($P < 0.05$) than that for Heat-WS. The ATTD of GE for C-WS was lower ($P < 0.05$) than that for Predigested-WS. The DE and NE values for DDGS did not differ from those of C-WS. The DE and NE values for C-WS were greater ($P < 0.05$) than those of Heat-WS, but lower from those of Predigested-WS.

4.4. DISCUSSION

The objective of this study was to determine the effect of heat pretreatment or enzymatic predigestion of WS on SID of AA and energy value of the resulting DDGS. Conventional corn DDGS was included for comparison. The starch, NDF, ADF, CP, EE and AA contents of the DDGS used in the current study were comparable with those previously reported in the literature for low-oil corn DDGS (Stein and Shurson, 2009; Espinosa and Stein, 2018; Espinosa et al., 2019). The DDGS and C-WS fed in the current study were similar in all components that were analyzed in the current study except for the NDF content, which was higher in C-WS than DDGS. The EE content in C-WS was lower than that reported by Han and Liu (2010) and Zangaro et al. (2018) for the WS. The reason for the lower content of EE in WS than expected is unclear. The CP content and AA composition of C-WS were similar to those reported by (Han and Liu, 2010) and Yang et al. (2017) for WS. In the current experiment, WS was pretreated at 140 °C and 70 psi for 15 min with the goal of disrupting fiber components, and thereby improving nutritional value of the WS. However, heat pretreatment of WS resulted in reduced the CP and AA contents and increased ADF and ash contents. Heat damage of feedstuffs result in increased analyzed ADF content in the feedstuffs (Almeida et al., 2013). As previously mentioned, the heat pretreatment of WS can be considered as a form of LHW pretreatment in which no additional water needs to be added to the biomass. The purpose of LHW pretreatment is to solubilize hemicelluloses and improve the digestibility of cellulose. The hemicellulose content of a feedstuff is estimated as the difference between NDF content and ADF content of the feedstuff (Rahman et al., 2017). Thus, LHW is expected to reduce the hemicellulose content of the pretreated feedstuff without significant effect on the cellulose content. In Heat-WS the ADF level resulted considerable higher and the NDF levels resulted like those

of C-WS, this might be the result of a lower hemicellulose content. Thus, a possible explanation to the observed increase in ADF could be attributed to the detection of products captured as ADF whereas the unchanged NDF value could indicate hemicellulose depletion, as the value of NDF did not increase with the rise in ADF content. Furthermore, heat treatment of feedstuffs causes Maillard reactions, which reduce the concentration and digestibility of Lys and other AA (Fontaine et al., 2007). Thus, the reduction of CP and AA levels on Heat-WS were the result of the heat pretreatment applied to WS, moreover, the observed considerable increased on ash content further indicates overheating. In contrast, predigestion of WS resulted in its increased CP and AA contents, but without significant effect on its NDF and ADF content. Jakobsen et al. (2015) observed increased concentration of CP of DDGS fermented with a mixture of cellulase and xylanase, and attributed it to the depletion of sugars (that were otherwise diluting the CP and AA) in the fermented DDGS. Bals et al. (2006) similarly observed reduction in concentration of CP in DDGS due to enzymatic hydrolysis of the latter with xylanase and cellulase. Thus, the increase in CP and AA contents in WS due to pretreatment could be attributed to depletion of sugars in the WS be the predigestion.

The AID of CP and AA values of conventional DDGS were comparable to those reported by Soares et al. (2012) and Stein and Shurson (2009). In the current study, the AID of CP and AA values for C-WS were higher than those for corn DDGS. As previously mentioned, DDGS is subjected to high temperatures (~500°C) during the drying stage of its production (Liu and Rosentrater, 2011), which in turn can affect the digestibility of CP and AA (Almeida et al., 2013; Lyberg et al., 2013). The C-WS fed in the current study was dried under mild conditions (less than 100 °C) and therefore the differences between C-

WS and corn DDGS with regard to the digestibility of CP and AA of may be due to differences in the amount of heat that was applied to the feedstuffs. The AID of CP and AA values for the C-WS fed in the current study were greater than the values that were reported by Yang et al. (2019). In the study of Yang et al. (2019), diets were fed in liquid form, whereas in the current study, diets were fed in dry form. Thus, the discrepancy between the current study and that of Yang et al. (2019) in AID of CP and AA could be explained by the differences in forms in which diets were fed. The observed SID of AA of DDGS were comparable to those reported by Espinosa et al. (2019) for low-oil DDGS. The SID of most AA for C-WS were greater than those for conventional DDGS. The observed values of SID of AA were comparable to those reported by Yang et al. (2019). The AID and SID of AA (especially Lys) values for Heat-WS were lower than those for C-WS. Previous studies have shown that Lys digestibility can be severely affected by heat treatment of feedstuffs as consequence of Maillard reactions (Martinez-Amezcua et al., 2007; Almeida et al., 2013). Hence, the observed reduction in digestibility of AA due to heat pretreatment may have been due to heat damage. The observed reduction in the AID of DM due heat pretreatment can be explained by the observed reduction in digestibility of AA. Predigestion of WS increased on AID of DM, OM and GE, but did not affect AID of CP and AA and SID of AA. Some NSP degrading enzymes have been used in the past in some ethanol plants to enhance oil recovery from WS (Luangthongkam et al., 2015) because oil bodies can be trapped between NSP and protein matrix. Therefore, the observed increased on AID of DM, OM and GE for Predigested-WS was likely due to depolymerization of NSP components caused by incubation with enzymes, which lead to

exposure of entrapped nutrients to digestive hydrolysis. It is not clear why the digestibility of CP and of most AA was not affected by the enzymatic predigestion.

The ATTD of DM and GE values for DDGS fed in the current study were comparable to the values reported by (Moran et al., 2016; Espinosa et al., 2019) for corn DDGS. The AID of GE of corn DDGS fed in the current study was comparable to that reported by Gutierrez et al. (2014b) for corn DDGS. The AID of GE and ATTD of GE of C-WS were similar to those of corn DDGS. Likewise, the DE and NE values of DDGS were comparable to values previously reported (Gutierrez et al., 2014a; Espinosa et al., 2019) for corn DDGS, and did not differ from those of C-WS. Incubation of WS with multienzyme increased its the DE and NE values of WS, whereas heat pretreatment of WS reduced its DE and NE values. The increase in DE and NE values of WS due to enzymatic predigestion can be explained by the increase in AID of GE and hence ATTD of GE by the predigestion. The NSP-degrading enzymes can hydrolyze NSP into sugars that are highly digestible. Also, the hydrolysis of NSP by the NSP-degrading enzymes can result in release of NSP-encapsulated energy-yielding nutrients, leading to increased energy digestibility.

4.5. CONCLUSIONS

The SID of AA, DE and NE values for DDGS fed in the current study were within the range of previous values reported for low-oil DDGS. The SID of AA values for C-WS were higher than those of corn DDGS. Heat pretreatment of WS negatively affected SID of AA, whereas predigestion of WS with multi-enzyme product that contained xylanase, celullase, α-galactosidase, resulted in higher SID of Lys. Furthermore, predigestion of WS increased DE and NE value of WS, while the opposite was observed as consequence of heat pretreatment of WS. Therefore, enzymatic predigestion can be an attractive

technology to increase the nutritive value of corn DDGS for pigs. On the other hand, heat pretreatment of WS with the conditions used in the current study negatively affected the nutritive value of WS, thus there is still need for determination of large scale conditions for pretreatment that can enhance the nutritive value of WS.

4.6. LITERATURE CITED

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Table 4.18. Ingredient and analyzed composition (on dry matter basis)

Item	*DDGS*	*C-WS*	*Heat- WS*	*Predigested-WS*	*NF*
Ingredient, % as fed					
Control WS	-	35.00	-	-	-
Corn DDGS	35.00	-	-	-	-
Heat-pretreated WS	-	-	35.00	-	-
Predigested WS	-	-	-	35.00	-
Cornstarch	50.74	50.74	50.74	50.74	79.90
Sucrose	6.35	6.35	6.35	6.35	10.00
Soybean oil	1.91	1.91	1.91	1.91	3.00
Cellulose	1.91	1.91	1.91	1.91	3.00
Calcium carbonate	1.60	1.60	1.60	1.60	0.90
Dicalcium phosphate	1.40	1.40	1.40	1.40	1.60
Salt	0.50	0.50	0.50	0.50	0.50
Marker TiO2	0.40	0.40	0.40	0.40	0.40
Pig mineral premix[i]	0.15	0.15	0.15	0.15	0.15
Pig vitamin premix[ii]	0.05	0.05	0.05	0.05	0.05
Magnesium oxide	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	0.40
Analyzed nutrients, DM basis					
Moisture, %	9.40	8.66	8.21	7.65	9.39
Crude protein, %	11.69	11.95	10.61	14.06	0.65
Gross energy, kcal/kg	4507	4454	4235	4594	4181
Ether extract, %	1.16	1.7	3.38	1.75	1.27
NDF, %	13.29	20.23	19.18	17.92	1.39
ADF, %	5.98	8.29	16.04	7.99	2.51
Ash, %	5.45	7.16	9.8	5.44	4.73
Indispensable amino acids, %					
Arg	0.43	0.42	0.31	0.61	0.01
His	0.29	0.28	0.24	0.37	0.00
Ile	0.42	0.43	0.38	0.60	0.01
Leu	1.24	1.46	1.37	1.80	0.03
Lys	0.32	0.31	0.21	0.49	0.01
Met	0.24	0.27	0.25	0.32	0.01
Phe	0.53	0.58	0.53	0.77	0.01
Thr	0.40	0.39	0.35	0.53	0.01
Trp	0.08	0.09	0.07	0.16	0.02
Val	0.53	0.53	0.47	0.70	0.01
Dispensable amino acids, %					
Ala	0.78	0.85	0.77	1.03	0.01
Asp	0.70	0.68	0.54	1.00	0.02
Cys	0.22	0.25	0.22	0.29	0.01
Glu	1.80	2.06	1.92	2.65	0.03
Gly	0.44	0.42	0.36	0.54	0.01
Pro	0.88	1.00	0.94	1.21	0.01
Ser	0.46	0.48	0.45	0.64	0.01
Tyr	0.36	0.43	0.37	0.56	0.01

[ii]Provided (per kg of diet): 75 mg Zn as $ZnSO_4$, 75 mg Fe as $FeSO_4$, 7 mg Cu as $CuSO_4$, and 20 mg Mn as $MnSO_4$.

[ii] Provided (per kg of diet): 2,226 IU vitamin A, 340 IU vitamin D3, 11.3 IU vitamin E, 0.01 mg vitamin B12, 0.91 mg menadione, 2.04 mg riboflavin, 12.5 mg pantothenic acid, 11.3 mg niacin, 0.23 mg folic acid, 0.68 mg pyridoxine, 0.68 mg thiamine, and 0.04 mg biotin.

Table 4.19. Analyzed composition of feedstuffs (on dry matter basis)

Item	DDGS	C-WS	Heat- WS	Predigested - WS
Analyzed nutrients, DM basis				
Moisture, %	10.77	8.53	7.91	5.52
Crude protein, %	32.83	30.79	28.18	39.66
Gross energy, kcal/kg	5085	5215	4637	5344
Ether extract, %	4.52	4.56	5.67	4.48
NDF, %	39.79	51.01	52.21	53.79
ADF, %	17.88	19.66	44.7	20.68
Starch, %	7.07	6.52	2.33	3.38
Ash, %	4.87	2.31	13.55	2.06
Indispensable amino acids, %				
Arg	1.39	1.17	0.89	1.61
His	0.90	0.81	0.68	0.99
Ile	1.32	1.24	1.11	1.64
Leu	3.83	4.07	3.84	4.73
Lys	1.00	0.85	0.55	1.31
Met	0.73	0.73	0.67	0.87
Phe	1.60	1.63	1.49	2.03
Thr	1.22	1.07	0.92	1.37
Trp	0.22	0.21	0.13	0.30
Val	1.74	1.53	1.37	1.99
Dispensable amino acids, %				
Ala	2.38	2.33	2.14	2.69
Asp	2.14	1.90	1.49	2.67
Cys	0.76	0.75	0.73	0.85
Glu	5.00	5.53	5.06	6.68
Gly	1.39	1.12	0.99	1.46
Pro	2.67	2.76	2.56	3.15
Ser	1.28	1.18	1.05	1.45
Tyr	1.20	1.18	1.12	1.40

Table 4.20. Apparent ileal digestibility CP and AA of corn WS (C-WS), corn DDGS, heat pretreated WS (Heat-WS) and predigested-WS fed to the growing pigs

Item, %	DDGS	C-WS	Heat -WS	Predigested - WS	SEM	*P* value
CP	56.22^{bc}	66.38^{a}	49.02^{c}	64.42^{ab}	4.19	0.0057
Indispensable AA, %						
Arg	74.04^{b}	82.9^{a}	55.41^{c}	85.61^{a}	4.403	<.0001
His	74.04^{b}	82.9^{a}	55.41^{c}	85.61^{a}	4.403	<.0001
Ile	74.24^{b}	87.35^{a}	75.63^{b}	88.88^{a}	1.781	<.0001
Leu	82.75^{b}	92.26^{a}	82.03^{b}	93.32^{a}	1.402	<.0001
Lys	62.71^{b}	76.15^{a}	42.9^{c}	78.88^{a}	3.58	<.0001
Met	81.07^{b}	91.69^{a}	84.14^{b}	93.05^{a}	1.325	<.0001
Phe	79.76^{b}	90.16^{a}	81.57^{b}	91.41^{a}	1.498	<.0001
Thr	63.04^{b}	78.39^{a}	64.97^{b}	81.64^{a}	2.719	<.0001
Trp	68.64^{b}	79.38^{a}	67.05^{b}	80.52^{a}	3.158	0.0027
Val	69.58^{b}	83.9^{a}	69.47^{b}	86.15^{a}	2.193	<.0001
Dispensable AA, %						
Ala	72.5^{b}	85.39^{a}	74.84^{b}	87.11^{a}	2.235	<.0001
Asp	65.69^{b}	81.22^{a}	56.51^{c}	81.75^{a}	2.832	<.0001
Cys	69.24^{b}	81.82^{a}	60.77^{c}	84^{a}	2.294	<.0001
Glu	77.11^{b}	89.43^{a}	77.44^{b}	90.56^{a}	1.776	<.0001
Gly	38.92^{b}	53.95^{a}	35.53^{b}	55.88^{a}	5.676	0.0002
Ser	72.35^{b}	84.01^{a}	73.37^{b}	86.34^{a}	2.136	<.0001
Tyr	78.95^{b}	89.61^{a}	83^{b}	91.15^{a}	1.673	<.0001

^{abc} Means within a row with similar superscripts are not different at $P < 0.05$.

Table 4.21. Standardized ileal digestibility of AA for WS (C-WS), corn DDGS, heat pretreated WS (Heat-WS) and predigested-WS fed to the growing pigs

Item, %	DDGS	C-WS	Heat - WS	Predigested - WS	SEM	*P* value
Indispensable AA, %						
Arg	85.66 [a]	87.09 [a]	60.74 [b]	87.79 [a]	4.394	<.0001
His	79.87 [b]	84.26 [a]	64.04 [c]	84.17 [a]	2.758	<.0001
Ile	77.55 [b]	84.66 [a]	63.58 [c]	85.27 [a]	2.948	<.0001
Leu	84.9 [bc]	91.89 [a]	75.55 [c]	88.8 [ab]	2.980	0.0059
Lys	67.35 [c]	75.47 [b]	53.93 [d]	84.07 [a]	3.005	<.0001
Met	85.63 [bc]	89.44 [a]	80.71 [c]	88.68 [ab]	2.832	0.0407
Phe	81.66 [b]	88.65 [a]	72.96 [c]	87.05 [a]	3.003	0.0033
Thr	72.29 [b]	77.52 [a]	57.76 [c]	81.85 [a]	3.378	<.0001
Trp	75.53 [b]	84.77 [a]	71.07 [b]	89.53 [a]	2.522	<.0001
Val	77.98 [b]	84.63 [a]	59.56 [c]	86.35 [a]	3.256	<.0001
Dispensable AA, %						
Ala	78.85 [b]	85.7 [a]	67.79 [c]	84.7 [a]	3.432	0.0011
Asp	70.97 [b]	79.09 [a]	58.23 [c]	82.19 [a]	3.096	<.0001
Cys	71.95 [b]	81.78 [a]	59.99 [c]	80.87 [a]	3.353	0.0002
Glu	81.18 [b]	90.38 [a]	73.17 [c]	88.51 [a]	3.012	0.0017
Gly	75.84 [bc]	85.96 [ab]	69.58 [bc]	93.01 [a]	6.613	0.0232
Ser	79.04 [b]	85.43 [a]	70.5 [c]	85.99 [a]	3.150	0.0024
Tyr	82.98 [b]	88.4 [a]	73.47 [c]	87.62 [a]	0.962	0.0014

[abc] Means within a row with similar superscripts are not different at $P < 0.05$.

Table 4.22. Apparent ileal digestibility (AID) of DM, OM, GE and Apparent total tract digestibility (ATTD) of DM, OM, CP, GE, NDF, ADF of corn WS (C-WS), corn DDGS, heat pretreated WS (Heat-WS) and predigested-WS diets fed to the growing pigs

Item	*DDGS*	*C-WS*	*Heat -WS*	*Predigested - WS*	*SEM*	*P* value
AID, %						
DM	71.08^{b}	72.27^{b}	61.72^{c}	78.53^{a}	2.277	0.0003
OM	72.53^{b}	73.16^{b}	65.05^{c}	79.54^{a}	2.18	0.0009
GE	74.82^{b}	74.45^{b}	66.35^{c}	81.49^{a}	1.967	0.0003
ATTD, %						
DM	81.82^{b}	83.2^{ab}	77.87^{c}	84.75^{a}	1.545	0.0007
OM	84.13^{ab}	84.41^{ab}	83.02^{b}	85.88^{a}	1.515	0.1602
CP	66.3^{b}	74.17^{a}	57.21^{c}	70.41^{ab}	3.383	0.0016
GE	83.82^{ab}	84.44^{a}	81.55^{b}	86.16^{a}	1.518	0.0172
NDF	38.45^{b}	57.75^{a}	57.81^{a}	60.91^{a}	8.448	0.0101
ADF	6.57^{b}	44.99^{a}	45.21^{a}	51.17^{a}	8.036	0.0044

^{abc} Means within a row with similar superscripts are not different at $P < 0.05$.

Table 4.23. Apparent ileal digestibility (AID) of GE, apparent total tract digestibility of DM, GE and digestible energy (DE) and net energy (NE) of feedstuffs

Item	*DDGS*	*C-WS*	*Heat-WS*	*Predigested-WS*	*SEM*	*P value*
AID of GE, %	50.09[b]	44.66[b]	26.56[c]	58.38[a]	5.419	<.0001
ATTD of GE, %	70.92[b]	75.21[b]	60.43[c]	79.15[a]	2.001	0.0004
ATTD of GE, %	73.3[b]	75.71[b]	67.15[c]	80.05[a]	2.549	0.0091
DE, kcal/kg DM	3440[b]	3687[b]	2665[c]	4068[a]	116.4	<.0001
NE, kcal/kg DM	2668[b]	2793[b]	1992[c]	2814[a]	81.9	<.0001

[abc] Means within a row with similar superscripts are not different at $P < 0.05$.

GENERAL DISCUSSION

The purpose of this study was to investigate optimal conditions for pretreatment and predigestion of corn WS for enhancing nutritional value of the resulting DDGS for pigs. Studies have previously been conducted on use of heat pretreatment and enzymatic predigestion technologies to increase the availability of carbohydrates in lignocellulose for ethanol production. However, limited information is available on the use of these pretreatment and predigestion technologies for enhancing nutritive value of fibrous feedstuffs for livestock feeding. Corn DDGS is available in large quantities for livestock feeding; however, the utilization of the DDGS by pigs is limited due to the high fiber content in the DDGS. Thus, the pretreatment and predigestion technologies can potentially be used to increase the nutritive value the DDGS for pigs. The cost of the technologies can be minimized by pretreating or predigesting WS at ethanol plants.

As previously described the efficiency of utilization of energy in corn DDGS is lower than that in corn grain, and this has been attributed to the higher content of DF in corn DDGS than in corn grain. Furthermore, NSP present in DDGS can further reduce utilization of other nutrients (Choct et al., 1996; Jha and Berrocoso, 2015). In addition to the negative effects of DF and NSP, quality of the protein in DDGS is often negatively affected by drying stage of producing the DDGS due to Maillard reactions (Almeida et al., 2013). Pigs, nevertheless, can utilize NSP as source of energy because part of NSP can be fermented in the hindgut to yield VFA. Previous studies have shown that, like in corn, high proportion of DF in DDGS is IDF, which is less fermentable than SDF (Jaworski et al., 2015). Furthermore, the most abundant NSP in corn DDGS are arabinoxylans, followed by cellulose (Jaworski et al., 2015). Cellulose is a polymer of glucose sugars that forms compact well organized structures

as part of the cell wall components. Whereas arabinoxylans form a net-like structure with a xylan backbone chain that is highly substituted by arabinose sugars that crosslink with ferulic acid and other components of lignin (Bach Knudsen et al., 2012). Heat pretreatment of lignocellulosic material is used in the bioethanol industry to produce ethanol from fibrous biomass. The purpose of the heat pretreatment is to disrupt the complex cell wall structure and depolymerize hemicellulose in order to generate fermentable sugars that can be transformed into ethanol. Therefore, it was hypothesized this technology can potentially be used to disrupt structure of dietary fiber and depolymerize hemicellulose in corn DDGS. The disruption of cell structure and depolymerization of hemicellulose in DDGS can result in release of NSP-entrapped nutrients, and generation of monosaccharides that can be digested or oligosaccharides that can fermented with net effect of an increase in energy digestibility.

Heat pretreatment and enzymatic predigestion of WS have been previously studied by Zangaro et al. (2018) using a porcine *in vitro* model; they observed increased digestibility and fermentability of WS due to the pretreatment. Heat pretreatment and enzymatic predigestion are attractive technologies because they can be integrated into ethanol production plants to minimize the cost of the technologies. In the current thesis research project, WS was heat pretreated and enzymatically predigested at various conditions to identify optimal conditions of heat pretreatment and enzymatic predigestion of WS for production of DDGS for pigs. Three experiments were conducted to achieve this goal.

In the first study (Chapter 2), the effects heat pretreatment of WS at various temperatures and for various durations were evaluated using a porcine *in vitro* technique. Several response criteria used in this study for determining the potential effects of heat pretreatment on energy and protein value of the resulting DDGS. Heat pretreatment reduced

the TDF content, and increased digestibility of DM and starch, and TGP of the WS, indicating that heat pretreatment increased energy digestibility and hence energy value of the WS. However, heat pretreatment reduced available Lys content of the WS, indicating that heat pretreatment reduced AA digestibility and hence the quality of protein in the WS. The optimal conditions of heat pretreatment of WS with regard digestibility of DM and TGP were temperature of between 140 and 160 °C and duration of between 20 and 30 min. However, the optimal conditions for heat pretreatment of WS with regard to available Lys content were temperature of about 101 °C and duration of 20 min. Therefore, the use of heat pretreatment technology to enhance energy value of DDGS can be limited by reduced availability of AA in the DDGS due Maillard reactions. This is evidenced by results from the in vivo study (Chapter 4) in which Lys content of heat pretreated WS was lower than that of untreated WS and conventional DDGS. Furthermore, the digestibility of Lys for heat pretreated WS was lower than that untreated WS or conventional DDGS. In the in vivo study, the DE and NE values for the WS were not improved by the heat pretreating WS despite the improvement *in vitro* digestibility of DM and TPG by the pretreatment in the first study. Heat pretreatment of WS used in the first study was performed at a laboratory scale, whereas heat pretreatment of WS used in the in vivo study was performed at a commercial scale in a research facility of an ethanol plant. The rate of cooling of a feedstuff that is heat pretreated on a large-scale is lower that of a feedstuff that is heat pretreated on a small-scale, implying that feedstuff that is heat pretreated on a large-scale is exposed to heat for longer period of time than feedstuff that is pretreated on a small-scale. Thus, the differences between the results in Chapter 2 and Chapter 4 with regard to the effects of heat pretreatment on energy value of WS can be attributed to differences in the scale of heat pretreatment of the WS.

In Chapter 3, untreated and heat pretreatment of WS were predigested with various multienzyme products to identify the best multi-enzyme for predigesting WS using a porcine *in vitro* technique. *In vitro* digestibility and fermentability of WS was enhanced by multi-enzyme predigestion. The greatest improvement in digestibility and fermentability of WS was observed when the WS was predigested with a multi-enzyme product that contained cellulase, xylanase and α-galactosidase. In the *in vivo* study, predigesting of WS with multi-enzyme increased digestibility of DM, GE and Lys of WS. Enzymatic pretreatment of lignocellulosic material has been used for depolymerization of cell wall components in order to generate fermentable sugars (Alvira et al., 2010). However, the effects of addition of enzymes, particularly xylanase and cellulose, to fibrous diets for pigs on nutrient utilization have been inconsistent. The inconsistent effects of the NSP-degrading enzymes in diets for pigs is attributed to limited time of interaction between the enzymes and their substrates in the GIT. Therefore, enzymatic predigestion technology can integrated in ethanol producing plants to enhance the nutritive of DDGS for pigs.

Overall, heat pretreatment and enzymatic predigestion of WS are attractive methods for enhancing the nutritive value of corn DDGS as evidenced by the results from studies of this thesis research project. However, large-scale heat pretreatment of WS *in vitro* at optimal conditions identified in the first study of this thesis research can negatively affect quality of protein in the WS and fail to improve energy value of the WS for pigs. Future research is needed to determine or investigate:

- Adaptation of optimal conditions of heat pretreatment of WS identified at laboratory scale for large-scale production heat pretreated DDGS in ethanol plants.

- The current research was done using whole stillage that did not undergo the conventional production process of production of corn DDGS (as described in chapter 1), therefore there is still need to determine nutritive value of enhanced DDGS that includes a multi-enzyme incubation of whole stillage in the process of production.
- Effects including pretreated or predigested DDGS in diets for pigs on growth performance and health status
- Economic viability of including pretreated or predigested DDGS in diets for pigs

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